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The Journal of

CLINICAL ENDOCRINOLOGY and METABOLISM

Official Journal of The Endocrine Society

VOLUME 16 NUMBER 8

AUGUST, 1956

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The Journal of
CLINICAL ENDOCRINOLOGY
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VOLUME 16 AUGUST, 1956 NUMBER 8
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METABOLISM OF ADRENAL STEROIDS IN DYING PATIENTS*†

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THE status of adrenocortical function and the metabolism of corticosteroids associated with impending death have not been well defined. The possibility exists that death may be associated with "exhaustion" of the adrenal cortex (1). It has been reported that the plasma potassium concentration of dying patients is higher than that seen in normal subjects (2, 3), a finding compatible with the presence of adrenal insufficiency. On the other hand, there is some evidence which suggests that there may be hyperfunction of the adrenal cortex at the time of death, although no rigorous proof of this has yet been submitted. Thus, it has been shown by sev-

Received December 9, 1955.

* This investigation was supported in part by research grants A-29 (C3) and A-2 (C7) from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, United States Public Health Service and by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under contract No. DA-49-007-MD-136.

† Some of the data were presented at the Annual Meeting of the American Federation for Clinical Research at Atlantic City on May 2, 1954, and have been published in abstract forms (*Clin. Res. Proc.* **2**: 83, 1954, and *J. Clin. Endocrinol. & Metab.* **15**: 893, 1955).

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eral investigators that severely ill or stressed patients excrete large amounts of 17-ketosteroids and corticosteroids in the urine (4, 5). Substantial neutrophilia and severe eosinopenia have been reported in dying patients and these changes in the blood have been interpreted to indicate hyperfunction of the adrenal cortex (6, 7). Since the evaluation of adrenal function has depended on such indirect indices, it is not surprising that so little is known concerning adrenocortical function in dying patients.

Previous studies in our laboratory have shown that high levels of plasma 17-hydroxycorticosteroids were present for several days before death in a wide range of serious illnesses (8, 9). The present study was undertaken to measure the function of the adrenal cortex and the metabolism of 17-hydroxycorticosteroids (17-OH-CS) in moribund patients, utilizing the somewhat more direct tests of these functions that have been developed in this laboratory.

METHODS

By the dying, moribund, terminal or agonal state (all these terms will be used interchangeably in this paper) is meant that irreversible condition which usually terminated in death within seventy-two hours. Some of the patients appeared moribund, and were designated so, many days before death, whereas other patients suddenly became critically ill and expired within a short period. Obviously, the diagnosis of the moribund patient is somewhat subjective and is based on the clinical impressions of the observer. Many of the patients diagnosed and studied as moribund survived for fairly long periods of time and were excluded from the present study.

Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels (10). The methods for the determination of urinary 17-OH-CS and 17-ketosteroids (17-KS) (11), and of plasma conjugated corticosteroids by chromatographic techniques (12) have been described. Methods previously published were used for the evaluation of adrenocortical capacity and rate of disappearance of exogenous hydrocortisone (cortisol) or tetrahydrocortisone (13, 14).

Radioactive hydrocortisone (4-C^{14}-hydrocortisone) was obtained from the Endocrinology Study Section of the National Institutes of Health. The mode of administration of 4-C^{14}-hydrocortisone and the determination of radioactivity of the various plasma and urinary fractions have been previously described (15). The 17-OH-CS levels in the normal subjects were determined on blood obtained between 7:00 and 9:00 a.m.—a time when the free steroid level is at its highest during the day. In contrast, the level in the moribund patients was determined on blood obtained at various times of the day or night.

RESULTS

In Figure 1 are shown the plasma 17-OH-CS levels in 66 moribund patients. The levels in these patients are compared to those of normal subjects and of patients with adrenal insufficiency or with Cushing's disease. It will be noted that none of the levels of 17-OH-CS in the dying patients falls within 2 S.D. of the average level in normal subjects (16). These samples were taken during a period ranging from as early as seventy-two hours

prior to death to a few minutes following death. The causes of death in these patients varied, and were the usual illnesses seen in a general hospital. Results of repeated determinations of the plasma 17-OH-CS level in several patients for varying periods preceding death, are listed in Tables 1 and 2. In general, there was a tendency for the highest 17-OH-CS levels in the plasma to be present shortly before, or at the time of death. In 2 cases, however, the maximum levels appeared to have been reached four days before exodus.

The possibility existed that the high plasma 17-OH-CS values, as measured by the Porter-Silber reaction, were due to non-steroid substances present in the blood of moribund patients. To test this hypothesis the plasma samples obtained from 7 terminal patients were each divided into two aliquots. On one aliquot the 17-OH-CS levels were determined by the method of Nelson and Samuels (10); on the other aliquot they were measured by a paper chromatographic technique (12). The average plasma 17-OH-CS level in these 7 patients as measured by the method of Nelson and Samuels was 50 μg./100 ml., and 34 μg./100 ml. by the paper chromatographic technique. It should be pointed out that the only 17-OH-corticosteroid found by the paper chromatographic technique was hydrocortisone. It appears, therefore, that the method of Nelson and Samuels does, in fact, measure predominantly hydrocortisone in the plasma of dying patients. The tetrahydro derivatives of the 17-OH-corticosteroids could not be detected in the paper chromatograms of the plasmas of these patients.

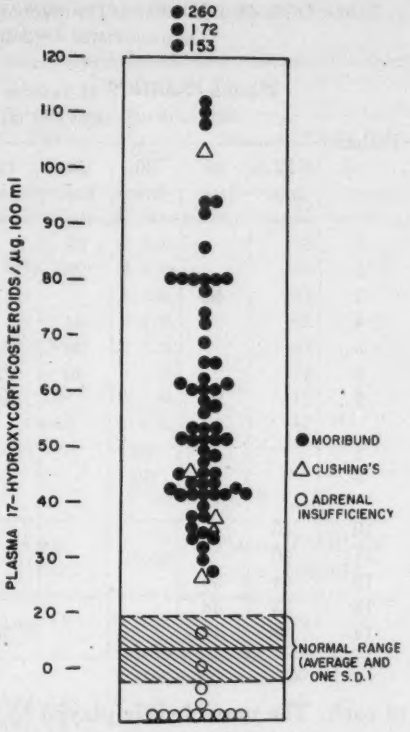

FIG. 1. Plasma 17-OH-CS levels in dying patients compared to those of normal subjects, patients with Cushing's disease and those with adrenal insufficiency. Three moribund patients had values above the range of the column.

The elevated levels of 17-OH-CS in terminal patients may result from increased secretion by the adrenal cortex or impaired metabolism of these steroids, or a combination of both. Studies were performed to test the role

TABLE 1. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN MORIBUND PATIENTS AT VARIOUS TIMES (±2–3 HOURS) BEFORE DEATH

Patient	Plasma 17-OH-CS at various times before death (μg./100 ml.)						Clinical condition
	72 hrs.	48 hrs.	36 hrs.	24 hrs.	12 hrs.	0	
1				68		72	Myocardial infarction
2				28		48	Acute leukemia
3		40				40	Cerebral vascular accident
4				44		50	Carcinoma of lung
5				36		48	Progressive muscular dystrophy
6				40	42	60	Myotonia dystrophica
7		30			38	40	Cerebral hemorrhage and polycystic renal disease
8			48			40	Cholemia
9			36			47	Chronic glomerulonephritis in uremia
10	45					48	Myocardial infarction
11	40					52	Carcinoma of lung
12	44	56				40	Chronic pulmonary fibrosis
13	33	36				55	Cerebral vascular accident
14	48	43			56	61	Aplastic anemia

of each. The possible role played by release of steroids from extra-adrenal tissues, if such exists, is difficult to evaluate and will not be considered in the present paper.

In Table 3 and Figure 2 are shown the changes in plasma 17-OH-CS levels in 9 agonal patients during the infusion of 25 I.U. of corticotropin (ACTH) over a six-hour period. In many of the patients listed in Table 3 the levels found before the administration of corticotropin were already in the range of the levels seen following maximal stimulation of the adrenal cortex by corticotropin in normal subjects (13). The changes observed during the intravenous administration of corticotropin to dying subjects varied. In some a normal or supernormal increase in 17-OH-CS occurred; in others very little, or only a transient, increase was seen. Nevertheless, a rise in the already elevated 17-OH-CS levels was noted in each patient. What part of these increases could be attributed to adrenocortical stimulation and what part to spontaneously rising levels in moribund patients is difficult to evaluate.

To test the role played by impaired metabolism of 17-OH-CS in the production of the high levels in dying patients, hydrocortisone was administered intravenously and the rate of disappearance of the steroid from the

TABLE 2. PLASMA 17-HYDROXYCORTICOSTEROID LEVELS IN DYING PATIENTS AT VARIOUS TIMES BEFORE DEATH

Patient No. 15

Date	Hour	Plasma 17-OH-CS (μg./100 ml.)
July 8	2 P.M.	21
July 9	10 A.M.	21
	5 P.M.	19
July 10	2 A.M.	24
	12 Noon	35
	2 P.M.	42
	4 P.M.	62
	6 P.M.	54
Death	8 P.M.	64

Patient No. 16

Date (1954)	Hour	Plasma 17-OH-CS (μg./100 ml.)
July 9	10 A.M.	18
	5 P.M.	27
July 10	2 P.M.	28
July 11	1 A.M.	52
Death	2 A.M.	41

Patient No. 17

Date (1954)	Hour	Plasma 17-OH-CS (μg./100 ml.)
July 7	8 P.M.	25
July 8	3 P.M.	30
July 9	5 P.M.	24
July 10	8 A.M.	22
	2 P.M.	20
July 11	11 P.M.	31
July 12	8 A.M.	25
July 14		
Death	8 A.M.	61

Patient No. 18

Date (1954)	Hour	Plasma 17-OH-CS (μg./100 ml.)
July 25	12 Noon	85
	2 P.M.	88
July 27	9 A.M.	50
	1 P.M.	52
	8 P.M.	44
July 28	11 A.M.	42
	8 P.M.	52
July 29	10 A.M.	47
Death	4 P.M.	47

Patient No. 19

Date (1954)	Hour	Plasma 17-OH-CS (μg./100 ml.)
Aug. 11	9 A.M.	52
	11 A.M.	55
	1 P.M.	50
Aug. 12	10 A.M.	42
Aug. 15		
Death	6 A.M.	40

Patient No. 20

Date (1954)	Hour	Plasma 17-OH-CS (μg./100 ml.)
Aug. 14	10 A.M.	8
	1 P.M.	12
	4 P.M.	17
	11 P.M.	17
Aug. 15	2 P.M.	25
Aug. 16		
Death	2 A.M.	35

TABLE 3. PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS (μG./100 ML.) DURING THE INTRAVENOUS INFUSION OF 25 I.U. OF CORTICOTROPIN (ACTH) OVER A SIX-HOUR PERIOD IN DYING PATIENTS

Patient	Plasma 17-OH-CS	Plasma 17-OH-CS at various times after beginning of ACTH infusion		
	Control	2 hrs.	4 hrs.	6 hrs.
21	50	103	107	93
22	68	90	84	89
23	65	70		75
24	80		98	110
25	50	79	85	99
26	59	70	71	72
27	42	77	74	71
28	35	54	60	107
Mean	56	78	83	90
Normal (young subjects)	10	26	34	42

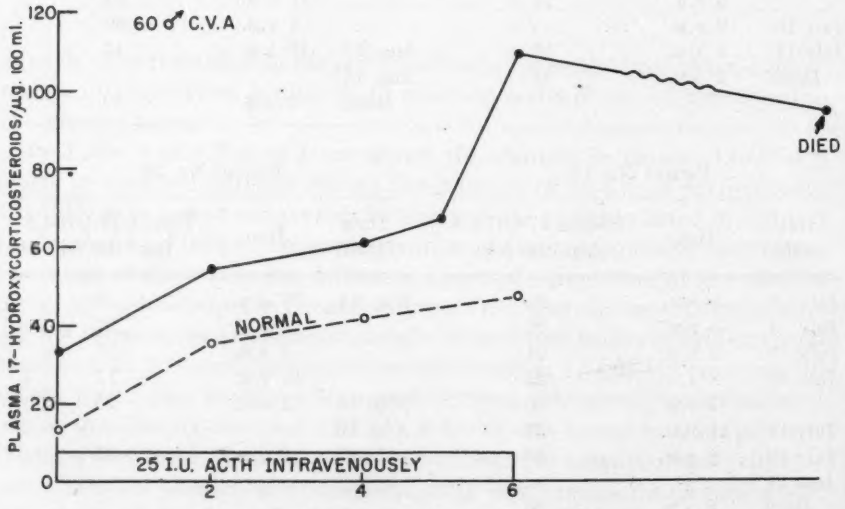

FIG. 2. Changes in plasma 17-OH-CS level during the infusion of 25 mg. of corticotropin (ACTH) in a moribund patient. The changes in a normal subject of comparable age are shown for comparison.

plasma was followed. The results in 7 patients are shown in Table 4. In Figure 3 the mean curve of these patients is compared with the normal curve. In all patients, as judged by the maintenance of very high plasma 17-OH-CS levels, there was definite impairment of metabolism of the administered hydrocortisone when compared to its rapid clearance from the plasma of normal subjects (17).

Since it was difficult to appraise spontaneously rising levels of 17-OH-CS in dying patients in the interpretation of the high levels following hydrocortisone administration, radioactive hydrocortisone (approximately 1 μc.

TABLE 4. LEVELS OF PLASMA 17-HYDROXYCORTICOSTEROIDS (μG./100 ML.) FOLLOWING THE INTRAVENOUS ADMINISTRATION OF THE FREE ALCOHOL OF HYDROCORTISONE (50 MG.) IN A SINGLE INJECTION TO DYING PATIENTS

Patient	Plasma 17-OH-CS	Plasma 17-OH-CS at various times after injection of hydrocortisone				
	Control	1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
29	172	420	360	300	320	
30	37	120	95	73		
31	27	152	138	110	80	
32	43	118	116	108	114	94*
33	108	180	206	187	174	
34	50	130	140	138	140	146
35	80	159	160	169	148	150
Mean	74	183	174	155	163	148
Normal (young) subjects	14	77	47	27	14	8

* Values 7 hours after injection.

of 4-C^{14}-hydrocortisone) was administered intravenously in 2 moribund patients. The disappearance of the radioactivity of the free steroid fraction in the plasma was much slower in the terminal patients, after an initial more rapid fall, than that in normal subjects and ultimately reached a plateau after sixty minutes (Fig. 4). In addition, the radioactivity of the plasma steroids conjugated with glucuronic acid, determined in only 1 of the dying patients, did not reach the high levels when compared to those of normal subjects. These findings, in general, are in agreement with those seen following the administration of non-radioactive hydrocortisone.

It has been shown that in normal individuals the preponderant part of the urinary corticosteroids is excreted in conjugated form, primarily as glucuronides, and only a minor part in the free form (18, 11). A study was

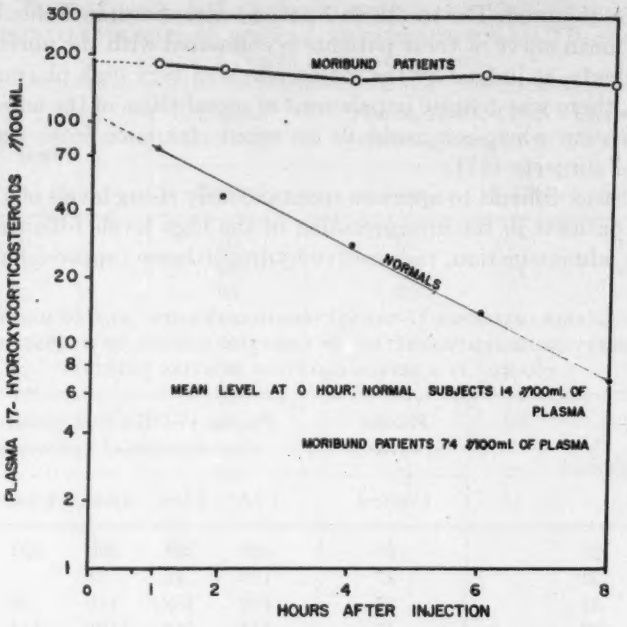

FIG. 3. Plasma 17-OH-CS concentration following the injection of 50 mg. of hydrocortisone intravenously into each of 12 normal subjects and 7 moribund patients. The levels depicted were obtained by subtracting the 17-OH-CS level at 0 hour. The dotted lines represent extrapolation of the curves to zero time.

made of the excretion of 17-OH-CS and 17-KS in the urine of 4 dying patients without evidence of previous renal disease (Table 5). In each case both the free and the conjugated 17-OH-corticosteroids were determined. The preponderant part of the 17-OH-CS in the urine of these patients was in the conjugated form. The amount of free steroid present in 2 of the patients did not differ from that seen in normal subjects, and in the other 2 patients the concentrations of steroid excreted relative to the total amount of free steroid fell well within the range seen in subjects after surgical stress (19). In the 4 moribund patients studied, the excretion of 17-OH-CS in the urine ranged from low to normal levels, in contrast to that in patients under surgical stress (19, 20).

To test further the role of the kidney, and the forms in which the metabolites of hydrocortisone are excreted in the urine, determinations were made of the radioactivity of the various fractions in the urines of the 2 patients given 4-C^{14}-hydrocortisone. It may be seen in Figure 5 that much less of the administered radioactive hydrocortisone appeared in the urines of the terminal patients. The ratios of the total radioactivity to that released

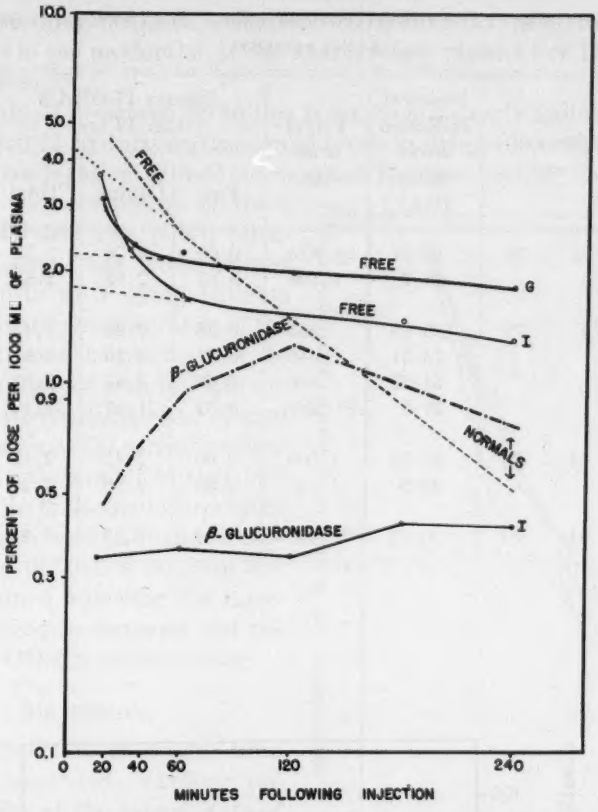

FIG. 4. Radioactivity in plasma following the injection of 4-C^{14}-hydrocortisone into 2 moribund patients. The "free" fraction of the plasma was obtained by determining the radioactivity of the chloroform-extractable fraction of plasma. The glucuronide fraction was obtained by determining the radioactivity of the chloroform-extractable fraction following incubation of the plasma with β-glucuronidase. The changes in the dying patients are compared to the mean value in 5 normal subjects. Note the rapid fall in the "free" radioactivity in the dying patients during the initial sixty minutes and the low glucuronide level in 1 patient. The "free" curves have been extrapolated to zero time.

by β-glucuronidase hydrolysis in the urine of 1 agonal patient did not differ significantly from those of normal subjects. Thus, after twenty-four hours the terminal patient excreted 22.8 per cent of the administered 4-C^{14}-hydrocortisone, with 13.7 per cent excreted as the glucuronide. Normal subjects excreted 82 per cent of the dose, with 44 per cent conjugated with glucuronic acid, during the same interval of time. The percentages of the total radioactivity excreted as free steroid were similar also. So far, the data

TABLE 5. URINARY 17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS IN 4 DYING PATIENTS

Patient	Sex and age	Period of collection before death (hrs.)	Vol. of urine (ml.)	Urinary 17-OH-CS (mg./12 hrs.)			Total 17-KS (mg./12 hrs.)
				Free	Conjug.	Total	
36	M 55	55–31	870	0.00	2.70	2.70	11.8
		31–7	1,190	0.03	2.82	2.85	12.6
37	F 72	123–99	285	0.25	2.20	2.45	1.47
		75–51	440	0.51	3.40	3.91	2.20
		51–27	480	0.48	3.45	3.93	2.00
		27–3	280	0.32	1.87	2.19	1.20
38	M 20	57–33	1,500	0.00	2.45	2.45	3.20
		33–9	1,160	0.30	3.90	4.20	3.16
39	M 70	36–12	440	0.39	6.55	6.94	1.58

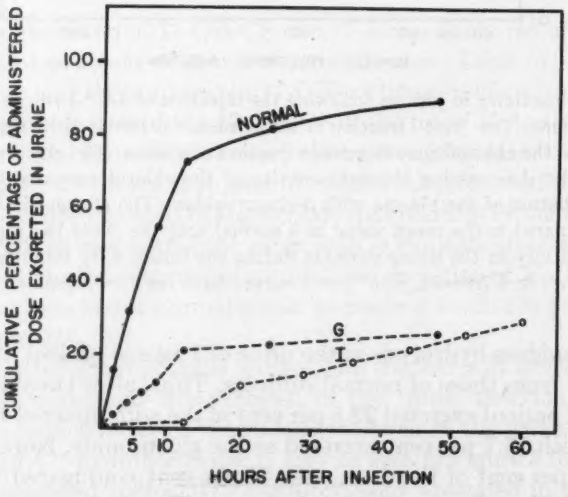

FIG. 5. Urinary radioactivity following the injection of 4-C^{14}-cortisol into 2 dying patients. The mean value in 5 normal subjects is drawn for comparison.

would seem to indicate that impaired renal excretion of steroids plays a minor role in the production of elevated levels of plasma free 17-OH-CS in dying patients.

The findings presented up to this point do not clearly indicate whether the elevated 17-hydroxycorticosteroid levels in dying subjects were due to impaired conjugation or reduced enzymic reactions during the reductive phase of the metabolism of these steroids. To test this point, tetrahydrocortisone was administered intravenously to 3 dying patients and the rate of disappearance of the compound from the plasma was observed. As may be seen in Figure 6, the tetrahydrocortisone was cleared from the plasma of the moribund patients at a rate similar to that in 2 normal subjects. It should be pointed out that each level in Figure 6 represents the difference between the value obtained following the injection of tetrahydrocortisone and the control 17-OH-CS concentration.

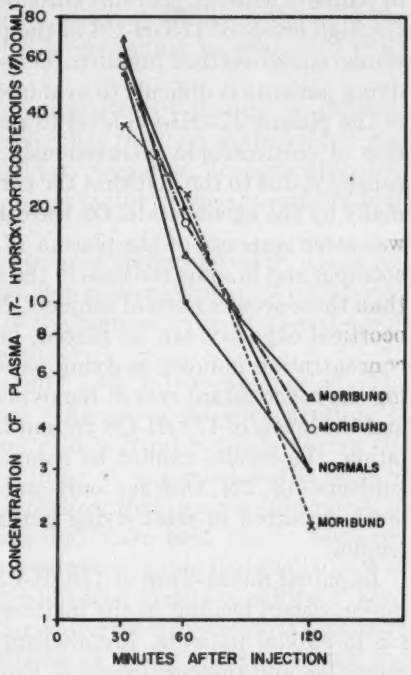

FIG. 6. Plasma 17-OH-CS levels in 3 dying patients and 2 normal subjects following the injection of 50 mg. of tetrahydrocortisone intravenously. The values were obtained by subtracting the 17-OH-CS concentration at 0 time from the concentrations observed following the injection of the tetrahydrocortisone.

DISCUSSION

It is difficult to ascertain, from the data presented, whether the irreversibility of the agonal state of the patients reported in this paper constitutes a different form of stress than conditions such as surgery, acute illnesses, and severe physical exertion. It is clear, however, that the effect of approaching death is generally greater, and the role of liver impairment more important. As a group, the moribund patients showed changes which were more consistent than those of other "stressed" groups of patients (14, 19, 21). Thus, it has been shown that elevated plasma 17-OH-CS levels were not invariably present in all patients during or following surgical stress (14, 19). The development of high plasma 17-OH-CS concentrations seemingly depended on the length, kind and site of operation and anesthesia. In general, the highest levels were seen

in patients in whom "life-threatening" situations developed (*e.g.*, hemorrhage and shock). In the dying patients, on the other hand, the plasma 17-OH-CS levels were invariably elevated and the metabolism of administered hydrocortisone was impaired in all patients studied. The latter was not true in all surgically stressed patients (14).

It is apparent that dying is not associated with adrenocortical exhaustion in patients without previous adrenocortical insufficiency. On the contrary, the high levels of 17-OH-CS in the plasma of these patients attest to continued adrenocortical function. The status of adrenocortical capacity in the dying patients is difficult to evaluate. The failure, in some of the patients, of the plasma 17-OH-CS level to rise significantly during the administration of corticotropin intravenously may indicate reduced adrenocortical capacity, due to the fact that the cortex already has been stimulated maximally by the agonal state. On the other hand, in most of the patients there was some response of the plasma 17-OH-CS level to stimulation by corticotropin and in some the rises in the steroid level were of greater magnitude than those seen in normal subjects. Not much reliance in evaluating adrenocortical capacity can be placed, however, on the changes in 17-OH-CS concentration induced in dying patients by corticotropin. Without knowing the concomitant rate of removal of the steroid, the distribution volume and the levels of 17-OH-CS present irrespective of the corticotropin stimulation, the results cannot be compared accurately with those in normal subjects (13, 22). One can only state that some rise in plasma 17-OH-CS levels occurred in most dying patients during administration of corticotropin.

Impaired metabolism of 17-OH-CS would seem to be a major, if not *the* major, defect leading to the maintenance of high plasma levels of 17-OH-CS in agonal patients. Metabolism of hydrocortisone probably involves reduction and then conjugation. From the data presented it would appear that the reductive reactions are limiting in the metabolism of hydrocortisone in terminal patients. The administration of tetrahydrocortisone (a compound very similar to tetrahydrocortisol) resulted in rapid clearance of the compound from the blood of moribund patients, indicating that conjugation is adequate. On the other hand, the intravenous administration of hydrocortisone, whether radioactive or not, resulted in slower disappearance of the free compound from the plasma of dying patients compared to that of normal subjects.

The kidney does not seem to play a major role in the elevation of the plasma 17-OH-CS in dying patients. The conjugated steroids were readily excreted by the kidney, at least in those patients without any evidence of previous renal disease, as indicated by the low levels of conjugated (as

glucuronides) steroids in the plasma of the patients and the presence of the steroids in the urine primarily in conjugated form, mainly as the glucuronides.

It has been reported that patients with liver disease do not metabolize administered hydrocortisone as readily as normal subjects do (23, 24), and that elevated plasma 17-OH-CS levels in patients undergoing surgical procedures are in part due to impaired liver function. Since liver function tests were not performed on the dying patients, the part the liver played in the causation of elevated plasma 17-OH-CS levels cannot be evaluated independently.

Evidence in the eviscerated rat (25), supported by findings in patients with liver disease (19) indicates, however, that the liver is the major, and perhaps only organ involved in glucuronide conjugation. It seems probable, then, that impaired liver function is a major factor in the abnormally high plasma levels of hormone.

Whatever the direct cause of these elevated steroid levels in dying patients, it would seem to become progressively more profound, as manifested by the occurrence, in most instances, of the highest levels of 17-OH-CS in the plasma at a time shortly before death.

Two observations of the present study seek fuller explanation. The more rapid fall, as compared to normal subjects, in the concentration of free 4-C^{14}-hydrocortisone in the plasma of moribund patients during the initial sixty minutes following injection, may be explained on the basis of an abnormally rapid diffusion from the plasma. Following equilibration in the tissues, high levels of these steroids may have been due to impaired metabolism. This explanation implies a larger miscible metabolic pool of free hydrocortisone in dying patients as compared to normal subjects. That the initial more rapid fall is not due to increased renal excretion is shown by the lesser excretion of 4-C^{14}-hydrocortisone in dying patients during the initial few hours. If the curves in Figures 3 and 4 are extrapolated to zero time, the apparent volumes of distribution of the injected non-radioactive cortisol in normal subjects and dying patients are equivalent to 50 liters and 25 liters of plasma respectively. On the other hand, if the straight-line portions of the radioactivity of the "free" curves obtained following radioactive cortisol injection are extrapolated to zero time, the apparent values of distribution of the injected 4-C^{14}-hydrocortisone are approximately 22 liters in the normal subjects and 50 liters in the moribund patients. It would appear, then, that in dying patients isotopic cortisol is distributed in a greater volume than is the excess hydrocortisone injected. The reason for these differences remains unknown.

The other observation needing explanation is the low-to-normal urinary excretions of 17-OH-CS and 17-KS in the dying patients. This is in con-

trast to surgically or medically stressed patients, who excrete increased amounts of steroids in the urine (4, 5, 19, 26). The lesser excretion in agonal patients is probably due to inability to hydrogenate 17-OH-CS, rather than to failure to conjugate, since free steroids are much less readily excreted by the kidney than are the conjugates. On the other hand, the increased urinary excretion of 17-OH-CS in surgically stressed patients is probably due in great measure to increased adrenocortical stimulation, since large amounts of conjugated steroids are found in the urines of these patients (19). Impaired metabolism of 17-OH-CS probably plays a secondary role in the elevation of the plasma steroid levels in these patients. Thus it would seem that the elevated plasma 17-OH-CS levels in dying patients are due to impaired metabolism of 17-OH-CS in the presence of continued production and secretion of steroids by the adrenal cortex at normal or reduced rates.

SUMMARY

1. Plasma 17-hydroxycorticosteroid levels have been determined in dying patients. In addition, the response of these levels to corticotropin was studied. The metabolism of 17-hydroxycorticosteroid in moribund patients was studied by observing the changes in the plasma 17-hydroxycorticosteroid levels following the administration of hydrocortisone. The metabolism of radioactive hydrocortisone was studied in 2 patients.

2. Dying was associated with elevated plasma 17-hydroxycorticosteroid levels. In most of the patients these levels could be further elevated by the intravenous administrations of corticotropin.

3. Intravenous hydrocortisone was cleared from the plasma of moribund patients at a much slower rate than that seen in normal subjects. The radioactive hydrocortisone (4-C^{14}-hydrocortisone) was initially cleared from the plasma at a faster rate than in normal subjects but thereafter was maintained at a plateau. These findings would seem to indicate that impaired metabolism of 17-hydroxycorticosteroids is a major factor in the elevated plasma levels in agonal patients.

4. Terminal patients excreted the preponderant part of 17-hydroxycorticosteroids in conjugated form. The total amount of 17-hydroxycorticosteroids excreted in the urine was less than that excreted by patients under surgical stress.

5. Dying patients cleared from the plasma the hydrogenated form of 17-hydroxycorticosteroids (tetrahydrocortisone) at a rate equivalent to that in normal subjects.

6. We interpret these results to indicate that the elevated 17-hydroxycorticosteroid levels in dying patients are due to impaired metabolism of these steroids in the presence of continued production and secretion of steroids by the adrenal cortex at normal or reduced rates.

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EFFECT OF INSULIN HYPOGLYCEMIA ON URINARY EXCRETION OF ADRENALINE AND NORADRENALINE IN MAN AFTER HYPOPHYSECTOMY

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IN A previous work it was shown that the intravenous administration of insulin in a dosage of 0.1 international unit per kilogram of body weight to healthy subjects caused a tenfold increase in the excretion of adrenaline, whereas the excretion of noradrenaline was not concomitantly increased (1). The aim of the present work was to examine the response of the adrenal medullary secretion to hypoglycemia in hypophysectomized patients.

MATERIAL AND METHODS

As shown in Table 1, the clinical material consisted of 20 patients (19 women and 1 man) from 33 to 65 years of age, who had been hypophysectomized because of metastatic carcinoma (breast, 19 cases; prostate, 1 case). The completeness of the hypophysectomy was checked by several tests of adrenocortical and thyroidal function. Only those patients were included in whom the tests demonstrated total hypopituitarism. However, small pituitary remnants may have been present even in these cases.

The insulin was administered intravenously at 8 a.m. in a dose of 0.1 I.U. per Kg. of body weight (except for the first test in Case 19, Table 1) with the patient fasting since the preceding evening. Capillary blood samples were taken 10, 20, 30, 45, 60, 75, 90, 150 and 180 minutes after the injection. The pulse rate was determined at regular intervals.

The patient emptied the bladder at 6 A.M. The urine was collected between 6 A.M. and 8 A.M. (control period), and between 8 A.M. and 11 A.M. (insulin tolerance test). For determination of free catechol amines the urine samples were prepared according to Euler and Orwén (2), and estimated by bioassay on the blood pressure of the cat and on the rectal cecum of the fowl (3).

RESULTS

The daily excretion of catechol amines after hypophysectomy

Figure 1 shows the excretion of adrenaline and noradrenaline before hypophysectomy and at different times after the operation. The preoperative level was normal, and with the exception of some increase during the second postoperative week (no tests were performed during the first week) a nor-

Received November 28, 1955.

TABLE 1. CASE MATERIAL AND RESULTS OF INTRAVENOUS TOLERANCE TESTS

Case No.	Patient	Sex	Age (yrs.)	Time after op. (mos.)	Substitution therapy		Insulin I.U.	Reaction*	Blood sugar (mg./100 ml.)							Pulse rate		Adrenaline (μg./min.)			Noradrenaline (μg./min.)		
					Cortisone (mg./day)	Thyroxine (μg./day)			Fasting	Lowest†/time (mg./min.)	45 min.	60 min.	90 min.	120 min.	150 min.	180 min.	Initial	Highest	z_1‡	z_2§	Diff. (z_2-z_1)	z_1‡	z_2§	Diff. (z_2-z_1)
1	F.F.	F	55	<1	0	0	6	++	109	50/30	50	52	53	57	65	67	83	100	2.6	4.6	+ 2.0	21	15	− 6
2	A.W.	F	40	<1	0	0	6	++	97	66/45	66	68	76	82	87	91	57	90	16	38	+22.0	21	12	− 9
3	L.O.	F	54	<1	0	0	7	++	93	41/30	43	43	56	56	54	60	86	86	4.5	14	+ 9.7	26	23	− 3
4	H.H.	F	43	<1	0	0	6	+	107	55/45	55	60	73	77	87	87	92	88	3.7	32	+28.3	36	82	+46
5	H.B.	F	44	<1	0	0	5	++	96	66/30	69	69	87	86	89	86	84	104	2.0	30	−28.0	6	22	+16
6	I.B.	F	43	<1	0	0	7		95	51/30	61	62	64	77	81	101			13	42	+19.0	14	11	− 3
7	T.A.	F	33	1	0	0	6	+	88	58/30	60	60	62	77	83	85	88	98	3.6	32	+28.4	6	15	+ 9
8	L.H.	F	60	1	0	0	5	+++	84	38/90	54	52	38	100 Gm. glucose			76	106	9.2	34	+24.8	27	71	+44
9	C.K.	M	65	1	50	0	7	(+)	112	70/45	70	79	83	87	114	105	58	74	1.6	17	+15.4	2	39	+37
10	H.B.	F	64	2	25	0	6		88	47/30	48	50	51	62	82	78			2.3	47	+44.7	9	35	+26
11	E.H.	F	45	3	25	0	5	+	101	54/30	65	66	78	78	82	96	71	88	1.5	7.4	+ 5.9	13	37	+24
12	E.P.	F	36	4	25	0	5	+	88	45/30	50	52	59	67	88	88	58	60	1.6	15	+13.4	25	22	− 3
13	E.B.	F	49	4	25	100	6	++	89	55/60	66	55	50 Gm. glucose						6.4	28	+21.6	6	28	+22
14	E.J.	F	49	4	0	100	6		98	55/45	55	57	56	58	58	58			3.8	4.2	+ 0.4	13	5	− 8
12	E.P.			5	0	0	5	(+)	110	73/30	89	89	97	95	101	107	88	90	4.3	60	+55.7	2	7	+ 5
15	L.N.	F	50	6	25	100	9	+	88	57/30	67	72	74	88	86	90	71	91	14	21	+ 7.0	27	34	+ 7
16	M.K.	F	33	6	25	100	6	+	91	56/30	66	69		89	95	105	66	74	1.5	45	+43.5	11	<1	−11
17	E.P.	F	43	7	25	100	7	+	95	54/30	61	61	69	77		81	81	88	2.5	17	+14.5	26	28	+ 2
18	M.B.	F	46	7	75	0	6	++	85	38/30	43	45	51	53	57	56	44	80	4.8	55	+50.2	32	<1	−32
19	L.G.	F	50	8	0	0	3	+	92	59/30	62	65	72	78	78	81	62	60	2.5	11	+ 8.5	27	13	−14
1	F.F.			10	25	0	7	++	90	34/30	40	40	42	51	58	61	74	85	3.6	42	+38.4	10	38	+28
3	L.O.			10	25	100	7	++	93	43/30	44	53	61		69	71	70	86	2.5	10	+ 7.5	23	16	− 7
3	L.O.			11	0	0	7	++	97	43/30	51	59	66	81	86	78	66	83	1.3	17	+15.7	3	24	+21
19	L.G.			14	25	100	7	+	99	57/45	57	59	73	82	106	145	58	58	3.3	30	+26.7	10	24	+14
17	E.P.			14	25	100	7	++	93	44/30	53	54	68	74		81	94	100	1.0	3.5	+ 2.5	25	20	− 5
7	T.A.			15	25	100	8	++	100	43/30	58	58				84	76	94	4.4	21	+16.6	58	34	−24
4	H.H.			17	25	0	7	++	95	48/30	54	65				89	65	72	9.1	18	+ 8.9	20	29	+ 9
9	C.K.			17	25	100	8	++	113	56/45	56	58	71	71	89	100	58	74	2.8	27	+24.2	18	23	+ 5
1	F.F.			20	25	0	8	++	102	40/30	42	44	45	49	58	64	78	87	2.3	42	+39.7	15	28	+13
20	A.L.	F	48	24	25	0	7	++	89	46/30	46	50					70	114	3.0	25	+22.0	16	23	+ 7

* + denotes a normal reaction as observed in healthy adults.
++ denotes a very marked reaction.
+++ denotes that the patient became comatose.
† The lowest blood sugar value obtained.
‡ Excretion during the control period—in most instances between 6.00 and 8.00 A.M.
§ Excretion during the three hours following insulin administration.

mal excretion was maintained after the operation. Administration of 25 mg. of cortisone daily generally did not alter the excretion values.

The excretion of catechol amines was followed daily in 4 cases after hypophysectomy (Fig. 2). An increased adrenaline excretion was observed in association with the administration of adrenaline (0.3 mg. subcutaneously). A marked increase was also observed in the 3 patients in whom the adrenaline excretion was measured during the day of operation or during the first postoperative day. In Patient G.E., adrenaline was also excreted in increased amounts during the second postoperative day, in association with a state of acute shock. Noradrenaline excretion was also markedly increased in Patient G.E. during the period of acute shock.

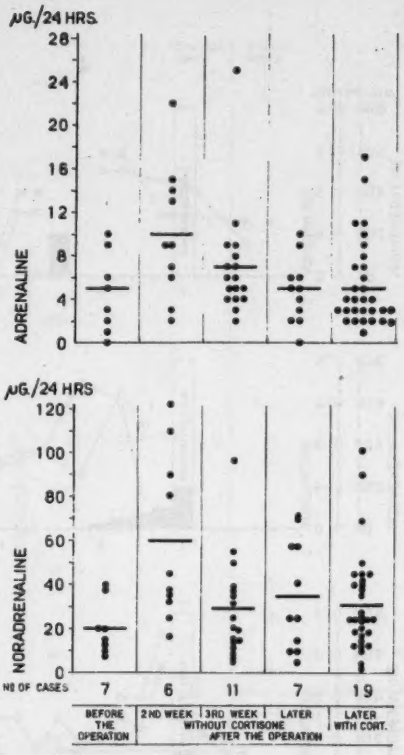

FIG. 1. Daily excretion of adrenaline and noradrenaline after hypophysectomy.

The excretion of adrenaline during rest, when measured during two hours before the administration of insulin (Fig. 3), was 4.5 ± 0.7 µg. per minute and thus not significantly different ($P > 0.2$) from the corresponding values in healthy subjects (5.5 ± 0.9 µg. per minute). The excretion of noradrenaline was 18 ± 2.2 µg. per minute (Fig. 4), and was not significantly different ($P > 0.2$) from corresponding values obtained under similar experimental conditions in healthy adults (12.3 ± 3.1 µg. per minute) (6).

Effect of insulin hypoglycemia on the excretion of catechol amines after hypophysectomy

Thirty *insulin tolerance tests* were performed in the 20 patients at different times after the hypophysectomy. Table 1 shows the dose of insulin given, and the changes in blood sugar level, pulse rate and excretion of adrenaline and noradrenaline. It also shows the substitution therapy employed, and the reaction of the patient to insulin. Reactions were classified as normal (+), stronger than normal (++), and precoma or coma

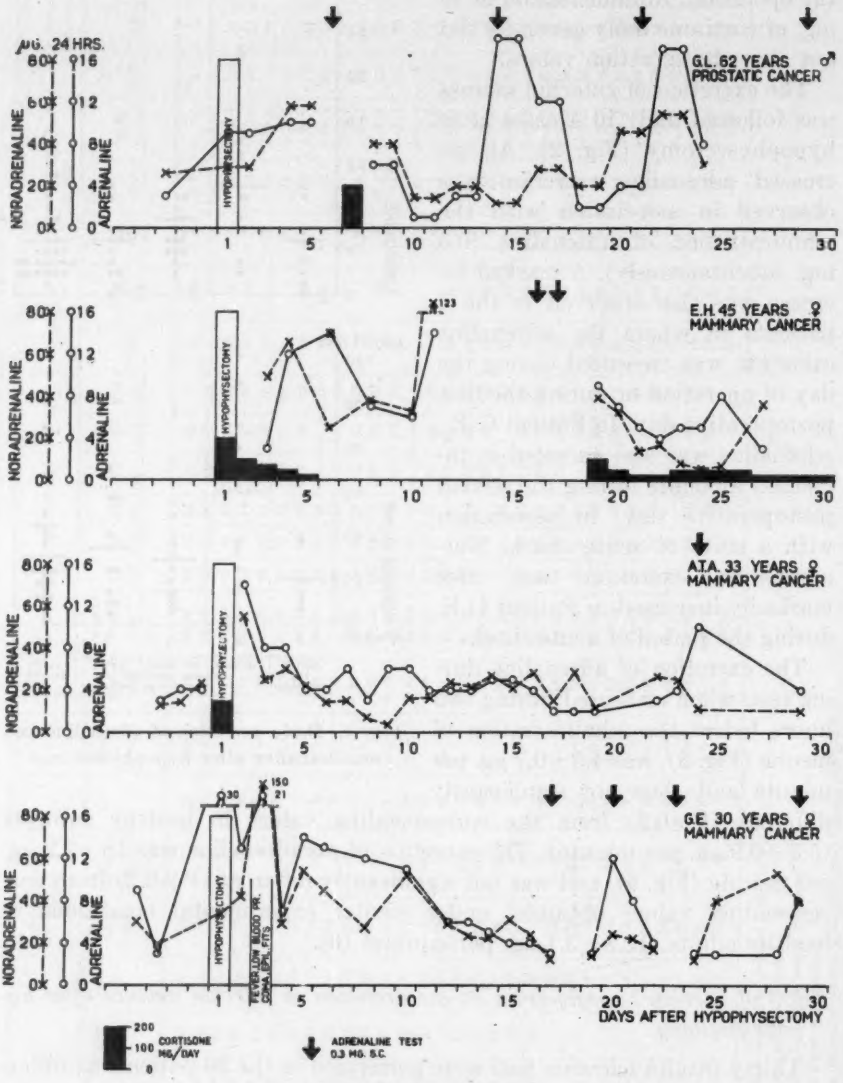

FIG. 2. Excretion of adrenaline and noradrenaline followed daily in 4 cases after hypophysectomy.

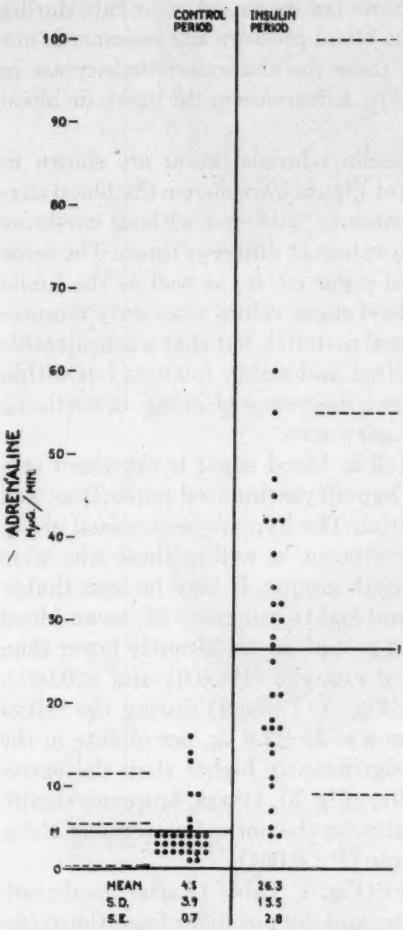

FIG. 3. Excretion of adrenaline during control period and after administration of insulin.

M denotes the mean value of the group of hypophysectomized patients; the actual figures are given at the bottom of the graph. The dotted lines denote the mean values and 2×S.D. limits in the normal material.

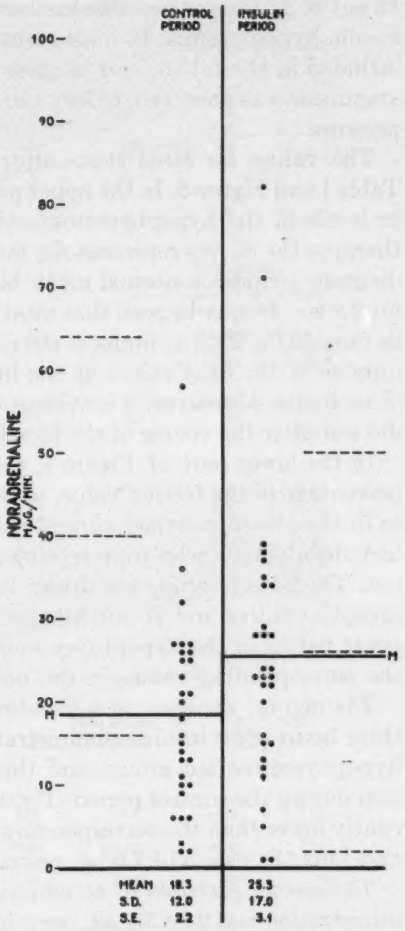

FIG. 4. Excretion of noradrenaline during control period and after administration of insulin.

M denotes the mean value in the group of hypophysectomized patients; the actual figures are given at the bottom of the graph. The dotted lines denote the mean values and 2×S.D. limits in the normal material.

(+++). It may be seen that a ++ or +++ reaction was observed in 16 out of 27 tests. The table further shows the increased pulse rate during insulin hypoglycemia. In most cases the blood pressure was measured (not included in the table), and in most of these the characteristic increase in amplitude was observed, chiefly caused by a decrease in the diastolic blood pressure.

The values for *blood sugar* after insulin administration are shown in Table 1 and Figure 5. In the upper part of Figure 5 are shown the blood sugar levels in the hypophysectomized patients, with and without cortisone therapy; the curves represent the mean values at different times. The same diagram includes a normal mean blood sugar curve, as well as the limits for 2×S.D. It may be seen that most blood sugar values after sixty minutes lie outside the 2×S.D.-limits of the normal material, but that a considerable number of the final values at one hundred and eighty minutes fall within these limits. Moreover, it is evident that a daily dose of 25 mg. of cortisone did not alter the course of the blood sugar curve.

In the lower part of Figure 5, the fall in blood sugar is expressed as a percentage of the fasting value, in the hypophysectomized patients as well as in the aforementioned normal material. The hypophysectomized group includes patients who were receiving cortisone, as well as those who were not. The 2×S.D.-limits are drawn for both groups. It may be seen that—except at thirty and at one hundred and eighty minutes—all mean blood sugar values in the hypophysectomized group are significantly lower than the corresponding values in the normal material ($P < 0.01$ and < 0.001).

The average excretion of adrenaline (Fig. 3, Table 1) during the initial three hours after insulin administration was 26 ± 2.8 μg. per minute in the hypophysectomized group, and thus significantly higher than the excretion during the control period ($P < 0.001$) (Fig. 3). It was, however, significantly lower than the corresponding value for the normal material of Euler and Luft (1), *viz.*, 55 ± 7.0 μg. per minute ($P < 0.001$).

The average excretion of noradrenaline (Fig. 4, Table 1) after insulin administration was 25 ± 3.1 μg. per minute, and did not differ from the excretion during the control period ($P > 0.2$). Moreover, it was almost identical with that found in the control group (26 ± 4.0).

DISCUSSION

The urinary excretion of adrenaline and noradrenaline provides a useful method of obtaining a relative measure of the production of catechol amines in the body. Studies in animals and man have shown that most of the adrenaline is derived from the adrenal medulla, whereas the largest part of the noradrenaline is released from the adrenergic nerves. Many of these studies deal with the catechol amines excreted in the urine, which

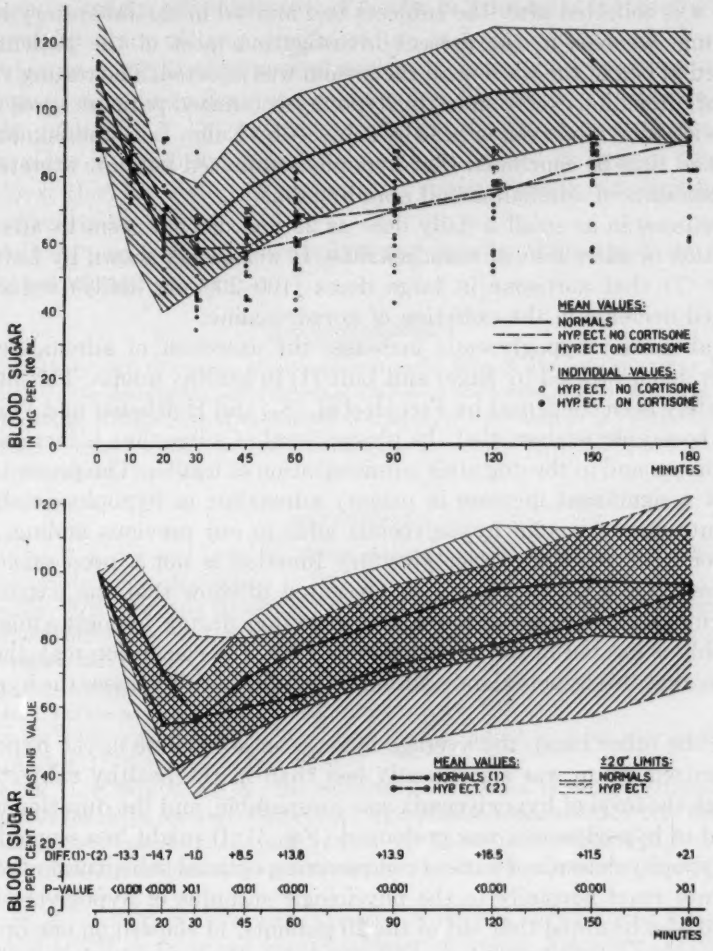

FIG. 5. Blood sugar level after administration of insulin (0.1 I.U. per Kg. of body weight).

represent only a small percentage of the total amounts released. On the other hand, the proportions of intravenously administered adrenaline and noradrenaline that are excreted in the urine seem to be fairly constant (4, 5).

The daily excretion of adrenaline and noradrenaline in the group of hypophysectomized patients was, on the whole, of the same magnitude as in a group of healthy adults (1). The higher noradrenaline values in this control group (40 ± 4.0 μg. per minute) are probably due to the fact that the

urine was collected after the subjects had arrived in the laboratory in the morning, whereas in the present investigation most of the patients remained in bed in the ward until the insulin was injected. The resting values for noradrenaline excretion in the hypophysectomized patients agree fairly well with those observed in healthy subjects by Euler, Luft and Sundin (6).

It can thus be concluded that hypophysectomized patients excrete normal amounts of adrenaline and noradrenaline.

Cortisone in as small a daily dose as 25 mg. did not seem to alter the excretion of adrenaline or noradrenaline. It was earlier shown by Luft and Euler (7) that cortisone in large doses (100–200 mg. daily) induced a marked decrease in the excretion of noradrenaline.

That insulin hypoglycemia increases the excretion of adrenaline was earlier demonstrated by Euler and Luft (1) in healthy adults. This finding has lately been confirmed by French *et al.* (8); and Holzbauer and Vogt (9) have been able to show that the plasma level of adrenaline is increased in the human and in the dog after administration of insulin. The present finding of a significant increase in urinary adrenaline in hypophysectomized patients during insulin hypoglycemia adds to our previous findings, and further indicates that intact pituitary function is not a prerequisite for this increase. Evidence has been presented to show that the mechanism governing the increase in adrenaline secretion during hypoglycemia presumably is located in the hypothalamus (10). The conclusion may then be drawn that this hypothalamic-adrenal medullary axis bypasses the hypophysis.

On the other hand, the average increase in adrenaline in the hypophysectomized group was significantly less than in the healthy subjects, although the level of hypoglycemia was comparable, and the duration of the period of hypoglycemia was prolonged (Fig. 5). It might be assumed that the hypophysectomized patient not receiving optimal substitution therapy does not react normally to the physiologic stimulus of hypoglycemia. It should also be noted that out of the 20 patients, 11 showed on one or more occasions an adrenaline value which was below the lowest in the previously studied normal group.

The excretion of noradrenaline was not altered in the hypophysectomized patients either before or during insulin hypoglycemia, indicating that the activity of the adrenergic nervous system is not influenced to any appreciable degree by hypophysectomy.

SUMMARY

In 20 hypophysectomized patients the daily excretion of adrenaline and noradrenaline was not altered except for some increase during the immediate postoperative period.

The intravenous administration of insulin in a dosage of 0.1 international unit per kilogram of body weight caused a hypoglycemia which disappeared more slowly than in normal subjects. The insulin hypoglycemia was accompanied by a significant increase in adrenaline excretion from a normal resting value of 4.5 ± 0.7 μg. per minute to 26 ± 2.8 μg. per minute. The latter value is significantly lower than that induced in normal controls, but shows that the ability to respond to hypoglycemia is not abolished by hypophysectomy.

Insulin hypoglycemia did not induce any significant change in the excretion of noradrenaline.

Acknowledgments

The authors wish to express their appreciation to Mrs. S. Hellner-Björkman for her assistance in the determination of catechol amines in the urine, and to Miss Eva Issén, Miss Ing-Mari Nilsson and Mrs. Kerstin Waldelöf for their help in the performance of the insulin tolerance tests. The cortisone used was generously provided by AB. Cibaprodukter, Stockholm, Sweden.

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EFFECT OF 2,4-DINITROPHENOL ON THYROID FUNCTION IN MAN*

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DINITROPHENOL has long been recognized as an agent capable of inducing hypermetabolism in various animal species by virtue of its uncoupling effect on oxidative phosphorylation within the Krebs tricarboxylic acid cycle (1, 2, 3). Thompson and associates (4) demonstrated a calorigenic effect of dinitrophenol in man, even when the subject was hypothyroid. In 1950 Wolff first described lowering of the serum protein-bound iodine (PBI) level in rats made hypermetabolic with 2,4-dinitrophenol (5). Radioactive iodine (I^{131}) studies failed to show inhibition of thyroidal iodine uptake or organification. Histologically, the pituitary and thyroid were also found to be normal following administration of 2,4-dinitrophenol (6). Goldberg *et al.* recently reported studies in rats which were interpreted as showing: a) decreased 24-hour thyroidal uptake of I^{131}, b) decreased rate of iodine organification, and c) increased disappearance rate of serum protein-bound iodine^{131} (PBI^{131}) from the circulation following administration of dinitrophenol (7). *In vitro* studies with sheep thyroid slices have shown that iodide transport across cell membranes can be inhibited by 2,4-dinitrophenol in concentrations adequate to reduce oxygen uptake (8).

We first recorded a fall in the level of serum PBI in man (9) coincident with a 2,4-dinitrophenol-induced elevation of the basal metabolic rate. It is our intent here to amplify that preliminary report and present further data in an attempt to explain the mechanism of this action.

METHODS

Eleven adults (9 men and 2 women) served as experimental subjects. Of these, 5 house staff members and 5 mental-disease patients had no known thyroid dysfunction

Received December 1, 1955.

* This study was supported by Grant No. 5A of the American Cancer Society and by Michigan Memorial Phoenix Project No. 99.

The dinitrophenol was made available through the courtesy of the National Aniline Division of the Allied Chemical & Dye Corporation, New York City.

or other physical disease. The eleventh subject, a staff physician, had had a surgical and a radioiodine total thyroidectomy performed for well-differentiated thyroid carcinoma, with resultant classic myxedema. He had been maintained in a euthyroid state on 3 grains (0.192 mg.) of desiccated thyroid per day for three years at the time of this study and was apparently free of carcinoma. The subjects ranged in age from 15 to 49 years.

The concentration of serum PBI (10) was determined daily in all subjects during a two-day control period, then during a two-day period while the subjects ingested 75 mg. of 2,4-dinitrophenol three times daily with meals, and then at least once during a one-day to three-day control period after dinitrophenol administration was stopped (Tables 1, 2, and 3). A fairly successful attempt was made to perform basal metabolic rate (BMR) determinations during each of these three periods in 4 of the subjects (Table 1). Daily serum cholesterol (11) and alkaline phosphatase (12) determinations were obtained on 2 subjects (Table 1). Eosinophil counts (13) and urinary 17-ketosteroid determinations (14) were performed daily on 2 subjects (Table 3). Thyroidal I^{131} uptake as a percentage of the administered dose, and quantitative 24-hour urinary and

TABLE 1. EFFECT OF 2,4-DINITROPHENOL (DNP) ON THE BMR AND ON SERUM PBI, CHOLESTEROL AND ALKALINE PHOSPHATASE LEVELS

Subject	Day No.	DNP (mg./day)	BMR (%)	Serum PBI (μg./100 ml.)	Serum chol. (mg./100 ml.)	Serum alk. phosph. (K.A. units/ 100 ml.)
C.B.	1		− 9	7.0	179	4.6
	2		−16	7.0	177	4.6
	3	225	+13	4.9	147	4.2
	4	225	+21	5.1	204	3.5
	5		+ 6	4.7	196	5.2
J.F.	1		+ 9	6.4	286	7.6
	2		—	6.1	248	8.9
	3	225	—	4.9	248	8.3
	4	225	+21	4.9	242	8.4
	5		+16	4.9	183	8.5
J.S.	1		− 9	5.5		
	2		—	5.1		
	3	225	—	5.1		
	4	225	+23	4.1		
	5		+28	4.1		
J.B.	1		−11	5.8		
	2		− 8	5.8		
	3	225	—	5.1		
	4	225	+30	4.7		
	5		—	4.7		

fecal excretions of I^{131} were determined in 2 subjects after ingestion of 100 microcuries of I^{131}. When these values had been followed daily for five days in these 2 subjects, an oral dose of 100 microcuries of I^{131} was again administered three weeks later, and the same determinations were then made daily for two days while the subjects were receiving dinitrophenol, and for two additional days after dinitrophenol administration was stopped (Table 3).

RESULTS

Fall in serum PBI level during dinitrophenol administration

The serum PBI level fell in every patient within forty-eight hours after the first dose of 2,4-dinitrophenol, the average decrease being 22 per cent. The mean value noted forty-eight hours after starting dinitrophenol differed from the mean control value by 1.3 gamma per 100 ml. a difference significant beyond the 1 per cent level when analyzed by the Student-

TABLE 2. EFFECT OF 2,4-DINITROPHENOL (DNP) ON SERUM PBI LEVEL IN 5 SUBJECTS

Day No.	DNP (mg./day)	Serum PBI level (μg./100 ml.)				
		A.E.	E.L.	D.K.	J.D.	S.S.
1		5.3	5.5	6.8	7.3	6.3
2		5.1	5.5	6.5	6.8	6.3
3	225	3.9	5.5	6.3	5.1	4.3
4	225	3.7	4.9	5.1	5.3	4.9
5		4.3	5.3	5.1	5.1	5.1

Fisher "*t*" method. The serum PBI values were lowered maximally within twenty-four hours in 6 patients, and within forty-eight hours in the remaining 5 patients (Tables 1, 2 and 3).

Rise in basal metabolic rate

In the 5 patients in whom basal metabolic rates were determined, the maximal values were observed forty-eight hours after the first dose of dinitrophenol in 4 patients, and at seventy-two hours in 1 patient (Tables 1 and 3). The average increase in the BMR was 29 per cent. The BMR was observed to decrease again within forty-eight hours after cessation of dinitrophenol in at least 2 patients (C.B., J.F., Table 1). No significant change was observed in basal pulse rates obtained at the time of the basal metabolic rates.

Serum cholesterol values

Serum cholesterol levels, determined in 2 patients (C.B., J.F., Table 1) as an additional index of the effect of thyroid hormone on body cells, showed no significant alteration.

TABLE 3. EFFECT OF 2,4-DINITROPHENOL (DNP) ON THE SERUM LEVEL CORRELATED WITH ROUGH INDICES OF ADRENOCORTICAL FUNCTION AND IODINE METABOLISM

Subject	Day No.	DNP (mg./day)	Serum PBI (μg./100 ml.)	BMR (%)	Eosinophils per cu. mm.	Urinary 17-KS (mg./24 hrs.)	Thyroidal uptake of I^{131} (% of dose)	Fecal I^{131} excretion (μc./24 hrs.)	Urinary I^{131} excretion (μc./24 hrs.)	Oral dose of I^{131}
C.W.C.	1						—	—	—	100 μc.
	2						40.7	0.87	64.7	
	3						40.2	1.22	6.1	
	4						40.1	0.10	0.7	
	5						—	—	0.2	
	6						40.9	—	—	
	1	—	5.8		411	—	—	—	—	—
	2	—	5.8		444	11.8	—	—	—	100 μc.
	3	225	4.3		622	11.3	23.4	0.35	49.0	
	4	225	4.1		500	11.2	27.4	1.86	5.4	
	5	—	3.7		466	13.1	28.1	0.20	0.9	
	6	—	—		—	—	28.1	—	0.4	
	7	—	4.9		—	—	—	—	—	
H.S.	1						—	—	—	100 μc.
	2						31.1	3.1	62.0	
	3						35.8	5.5	9.6	
	4						35.8	1.1	2.8	
	5						40.4	—	0.6	
	1	—	5.4	—	—	10.0	—	—	—	100 μc.
	2	—	5.3	−1	389	17.5	—	—	—	
	3	225	4.1	—	278	12.0	41.9	0.2	57.6	
	4	225	4.6	+30	278	9.2	40.8	6.2	5.14	
	5	—	4.5	—	411	11.8	47.5	1.4	0.9	
	6	—	4.9	—	—	15.3	45.0	—	0.4	

Serum alkaline phosphatase values

Serum alkaline phosphatase concentration was determined in Subjects C.B. and J.F. to see if there would be any observable change in these values during and after the period of dinitrophenol-induced uncoupling of oxidative phosphorylation. In spite of significant elevation of the BMR and a drop in the serum PBI level, no significant change in serum alkaline phosphatase values was observed in these 2 patients.

Indices of adrenocortical function

Because of statements in the literature regarding the inhibitory effect of adrenocortical steroids on thyroid function (15), blood eosinophil counts and 24-hour urinary 17-ketosteroid excretions were followed in Subjects C.W.C. and H.S. (Table 3) as a rough check on adrenocortical steroid activity during and after administration of dinitrophenol. No significant change was observed in these values.

Thyroidal I^{131} uptake and I^{131} excretion in urine and stools

Thyroidal I^{131} was followed to detect any effect of dinitrophenol in decreasing the rate of I^{131} uptake, or increasing the rate of I^{131} release from the thyroid. Urinary and fecal I^{131} excretions were followed to detect a possible increased rate of elimination of iodine as an explanation for the dinitrophenol-induced lowering of serum PBI concentration (Table 3). No significant change was observed in these values during or after administration of dinitrophenol.

DISCUSSION

We have demonstrated in humans a significant fall in the level of serum PBI and a coincident rise in the basal metabolic rate associated with the administration of 225 mg. of 2,4-dinitrophenol each day for two days. That these changes occurred in a subject who had no thyroid gland (J.B.) is evidence against the possibility that dinitrophenol exerts its effect on the serum PBI and BMR through the thyroid or pituitary glands. Thyroidal I^{131} studies (C.W.C. and H.S.) showing no consistent effect of dinitrophenol on I^{131} uptake are also evidence against this explanation. Urinary and fecal I^{131} studies in the same 2 subjects, showing no change following administration of dinitrophenol, suggested that accelerated excretion of iodine is not the cause of the decreased level of serum PBI. The conditions of this experiment probably would not allow us to detect an increased rate of release of I^{131} from serum PBI^{131} as a result of increased peripheral breakdown of thyroid hormone. The amount of I^{131} in PBI^{131} present in the blood stream at twenty-four to forty-eight hours would be too small, and too much of the I^{131} released would be recirculated through the thyroid gland.

The administration of comparatively massive dosage of dinitrophenol to rats over a relatively long period of time has been observed to alter the function of their thyroid glands, as shown by decreased 24-hour uptakes of I^{131} and decreased rates of iodine organification. These experimental conditions, however, are not comparable to the experimental conditions of our work.

The blood eosinophil counts and urinary 17-ketosteroid excretion values in Subjects C.W.C. and H.S. during and after administration of dinitrophenol constitute evidence that dinitrophenol does not exert the metabolic effects described here through the mechanism of altering adrenocortical steroid activity.

In view of the foregoing evidence that dinitrophenol does not act upon the thyroid or pituitary glands or by way of adrenocortical steroids to lower the serum PBI level and raise the BMR, one explanation might be that dinitrophenol accelerates peripheral utilization (or causes sequestration) of thyroid hormone. We have no direct proof that this is the mechanism.

SUMMARY

The oral administration of 225 mg. of 2,4-dinitrophenol per day for two days to 10 euthyroid adults and to 1 athyroid adult receiving maintenance thyroid medication resulted in a significant fall in the serum protein-bound iodine level and a rise in the basal metabolic rate. Evidence is presented to support the view that the mechanism of this action is not an effect of dinitrophenol on the thyroid or pituitary glands or on adrenocortical steroid activity. It is suggested that dinitrophenol increases the rate of peripheral utilization (or causes sequestration) of thyroid hormone.

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EFFECTS OF ESTROGENS ON RENAL HEMODYNAMICS AND EXCRETION OF ELECTROLYTES IN HUMAN SUBJECTS*

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IT HAS been generally accepted that estrogens cause retention of water, sodium and chloride. This belief has been based largely on the clinical observation that women gain weight and accumulate a certain amount of edema at times when the blood levels of estrogens are thought to be high, just before menstruation and during late pregnancy.

Thorn and his coworkers (1) recorded changes in weight in 50 normal subjects throughout the menstrual cycle. Of these, 24 gained 1 kilogram or more during the premenstrual period; 38 gained 1 kilogram or more at about the time of ovulation. Urinary excretions of sodium and chloride were determined in 2 of the patients who gained weight and in 4 control patients who did not gain weight. Retention of sodium and chloride was observed in the 2 subjects who gained weight, as well as in those who did not gain weight. The retention of electrolytes coincided with an increase in urinary levels of estrin as determined by bioassay in 2 subjects.

Thorn and his associates (2, 3) also studied the effects of exogenous estrogens on electrolyte excretion in dogs. They found that the excretions of sodium, chloride and water were diminished for twenty-four to seventy-two hours following subcutaneous injection of 5 mg. of estrogenic hormone. However, with continued administration of this hormone, electrolyte excretion reverted to pretreatment patterns.

White *et al.* (4) studied the effects of ovariectomy on the clearances of inulin and Diodrast in 2 dogs. No changes in the glomerular filtration rate or renal plasma flow were observed up to one year after ovariectomy.

Kenyon (5) reported that the administration of 5 mg. of estradiol ben-

Received December 1, 1955.

* This investigation was supported in part by a grant from the National Institutes of Health, Department of Health, Education, and Welfare, and in part by a grant from the Harbor Hospital Attending Staff Fund.

zoate to a dwarfish sexually immature girl induced an immediate but poorly sustained decrease in sodium excretion.

Dean, Abels and Taylor (6) studied the renal function of 4 women who had received 4 to 6 mg. of estradiol benzoate daily for from nine to twelve days. They found no change in glomerular filtration rate, renal plasma flow and tubular reabsorption of glucose. A depression of tubular reabsorption of ascorbic acid was noted in 3 subjects in whom that measurement was made.

It is evident from this brief review of the literature that the effects of sex steroid hormones on renal functions have not been properly assessed and that further investigation of this problem is warranted. The studies performed heretofore on human subjects have been few and have failed to include control data of the renal handling of water and electrolytes. The changes in weight during the menstrual cycle, upon which much reliance has been placed, can be due to a multiplicity of factors and cannot be accepted as an index of changes in renal function. Furthermore, in nearly all of the studies on the effects of exogenous doses of sex steroid hormones, the patients possessed active, intact ovaries—a factor which renders the interpretation of the data somewhat difficult.

The present studies were aimed at investigating the effects of sex steroid hormones on renal function. Since it has been shown recently that these hormones may be given intravenously, we thought that this route might afford an excellent technique for examining the acute effects of estrogen on renal hemodynamics and the excretion of electrolytes. The chronic effects of this hormone were studied in patients who received daily intramuscular injections of estrogen and on whom daily metabolic balances were determined. In order to exclude interference from the patient's own ovaries, all of these studies were carried out on postmenopausal women or on women whose ovaries had been removed surgically at least six months prior to the experimental procedure.

MATERIAL AND METHODS

Eighteen women were studied; 17 were post-menopausal and 1 had been surgically castrated several years before the assay. Ages varied from 32 to 76 years. All of the patients were at the hospital prior to and during the studies.

Three types of studies were made, as follows:

Experiment A was designed to study the effects of acute intravenous administration of estrogen and was carried out on 8 patients. Each was allowed to drink water freely on the day before the study. On the following morning breakfast was omitted but water was given orally or intravenously in the form of 5 per cent glucose solution. A priming dose of inulin and para-aminohippurate (PAH) was given intravenously over a five-minute period. This dose was calculated for each patient according to her body weight, the volume distribution of inulin being taken as 20 per cent and that of PAH as 40 per

cent of the body weight. Then followed a constant infusion of inulin and PAH aimed at maintaining plasma levels of approximately 200 mg. per liter for inulin and 30 mg. per liter for PAH. An equilibration period of twenty to thirty minutes was allowed, after which three to four control clearances of fifteen to twenty minutes' duration were performed. These clearances serve as controls. Thereafter, a dose of 25 or 50 mg. of estradiol-17β dissolved in 5 cc. of propylene glycol and mixed with 50 cc. of 25 per cent salt-poor human serum albumin was injected intravenously over one-minute to three-minute periods, and from three to five clearances of fifteen to twenty minutes' duration were performed. Hematocrit and serum protein levels were determined before, and several times after the injection of the mixture. In order to verify the estrogenic activity of the intravenously injected material, repeated vaginal smears from 5 patients were examined. Three daily smears were collected from each patient before the hormone injection. After the intravenous injection of estradiol, smears were taken at thirty minutes, sixty minutes, and every day thereafter for periods up to six days.

Experiment B was aimed at studying the effects of the vehicle alone, *i.e.*, human serum albumin and propylene glycol. This procedure was carried out on 5 patients, and followed a pattern similar to that in Experiment A.

Experiment C was designed to study the effects of repeated administration of estradiol on daily metabolic balances and was performed on 5 patients. All of these patients, though ambulatory, were confined to the hospital. All received a similar diet containing 85 mEq. per day of sodium as sodium chloride. Each patient was studied in three consecutive periods: The first period served as a control and lasted for three days. During the second period, estradiol-17β dissolved in butylene glycol was given intramuscularly in doses of 8 mg. a day for three to six days. The third period served as a post-treatment control and lasted for three to eight days. During all of these periods the patient's weight, blood pressure, intake and output were recorded daily. Vaginal smears were examined frequently before, during, and after the hormone injection. Urine was collected in 24-hour specimens, which were preserved with toluene and analyzed for sodium, potassium and chloride. The completeness of urine collections was checked by the creatinine excretion. Endogenous creatinine clearance was performed once before the estradiol was given and once during its administration. Glomerular filtration rate was roughly estimated from this clearance in these cases.

In all of the studies, inulin, PAH, sodium, potassium, chloride and creatinine levels in blood and urine were determined by methods described elsewhere (7).

RESULTS

Experiment A

Table 1 shows the results obtained in the 8 patients who received 25 mg. and 50 mg. of estradiol 17β dissolved in propylene glycol and mixed with human serum albumin. Each value before and after hormone administration represents the average of the collections made during this period. The urine flow in the control period varied from 1.6 cc. to 9.3 cc. per minute, with a mean of 5.4 cc. per minute. Following the intravenous administration of the estrogen mixture the urine flow did not change significantly. Similar results were observed in the values for renal plasma flow and glomerular filtration rate.

The renal handling of electrolytes showed a different pattern from that of renal hemodynamics. The concentration of sodium and chloride in the

urine fell in each instance. The average concentration of sodium during the control period was 47 mEq. per liter; this fell to 31 mEq. per liter. A similar fall was observed in the concentration of chloride. Potassium concentration showed a slight decrease following treatment with estradiol. The excretion of electrolytes (which can be calculated from Table 1) showed a trend similar to that of their concentration in the urine.

Figure 1 illustrates in detail a typical example of a patient who received 25 mg. of estradiol-17β mixed with 50 cc. of human serum albumin. Three control and three post-injection clearances were performed. It may be seen that the urine flow, renal plasma flow and glomerular filtration rate did not

TABLE 1. EFFECTS OF INTRAVENOUS ESTRADIOL MIXED WITH HUMAN SERUM ALBUMIN ON URINE FLOW (RPF), GLOMERULAR FILTRATION RATE (GFR), AND URINARY CONCENTRATION OF Na, Cl, and K. EACH FIGURE REPRESENTS THE AVERAGE OF THREE TO FIVE COLLECTION PERIODS, BOTH BEFORE AND AFTER THE INJECTION OF THE SOLUTION. ALL FIGURES ARE CORRECTED FOR BODY SURFACE AREA

Patient	Age (yrs.)	Dose of estradiol	UF		RPF		GFR		Na (mEq./L)		Cl (mEq./L)		K (mEq./L)	
			Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
S.T.	66	25 mg. i.v.	3.3	3.8	376	492	68.7	75.6	72.0	51.2	29.4	21.2	12.9	10.2
E.R.	70	25 mg. i.v.	4.7	5.7	503	570	64.1	63.2	24.2	14.0	19.4	8.7	8.2	4.3
E.O.	32	25 mg. i.v.	9.2	10.6	759	774	153.0	133.0	18.7	10.0	12.2	4.3	7.6	2.9
N.A.	71	25 mg. i.v.	7.2	5.0	333	314	87.7	81.7	34.1	19.8	22.5	10.1	7.2	5.8
S.B.	73	25 mg. i.v.	1.6	1.3	303	222	104.0	78.0	111.0	67.4	86.0	64.0	21.3	23.2
S.S.	65	25 mg. i.v.	2.6	2.6	442	285	90.0	84.0	56.0	46.5	32.0	19.0	10.5	10.3
E.H.	55	50 mg. i.v.	9.3	9.2	312	318	110.0	103.0	13.0	13.6	6.6	6.6	8.9	5.0
B.W.	67	50 mg. i.v.	5.5	7.0	515	487	109.0	101.0	47.5	25.0	30.5	14.0	7.1	4.9
		Mean	*5.4*	*5.6*	*443*	*433*	*88.2*	*89.9*	*47.0*	*31.0*	*30.0*	*18.5*	*10.5*	*8.3*

change significantly. However, both the excretion and the concentration of sodium fell progressively, beginning immediately after the injection of the estradiol mixture. Similar changes were observed in the behavior of chloride, but potassium excretion fell less markedly.

Experiment B

This experiment was aimed at studying the effects of the vehicle, which consisted of 5 cc. of propylene glycol and 50 cc. of 25 per cent salt-poor human serum albumin. Table 2 shows the results in the 5 patients who were studied in this manner. Here again the urine flow, renal plasma flow, and glomerular filtration rate did not change significantly after the injection of the albumin. However, the changes in the concentration of sodium, chloride and potassium in the urine were very similar to those observed after the injection of the mixture of estradiol and albumin. Figure 2 shows detailed data in a patient who exhibited the least response to intravenous injection of albumin and propylene glycol. The pattern of changes in renal

TABLE 2. EFFECTS OF THE VEHICLE ALONE—HUMAN SERUM ALBUMIN AND PROPYLENE GLYCOL—ON URINE FLOW (UF), RENAL PLASMA FLOW (RPF), GLOMERULAR FILTRATION RATE (GFR) AND URINARY CONCENTRATION OF Na, Cl, AND K.
(FOR DETAILS, SEE LEGEND OF TABLE 1)

Patient	Age (yrs.)	Preparation	UF		RPF		GFR		Na (mEq./L)		Cl (mEq./L)		K (mEq./L)	
			Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
O.H.	66	Albumin	10.4	6.6	333	368	110	94.7	65.5	35.0	45.5	26	15.5	10.7
L.J.	67	Albumin	4.5	5.7	—	—	—	—	27.0	14.2	14.5	10	16.4	8.1
B.L.	67	Albumin	5.4	5.0	440	427	97	90.0	45.0	38.0	35.0	27	11.2	11.1
M.J.	69	Albumin	5.5	7.0	494	532	129	107.0	27.0	20.0	24.0	17	9.8	7.1
O.L.	76	Albumin	6.0	6.3	237	248	64	63.0	74.0	48.0	66.0	42	13.0	11.0
		Mean	*6.3*	*6.1*	*376*	*393*	*100*	*88.6*	*47.8*	*31.0*	*37.1*	*24*	*13.1*	*9.6*

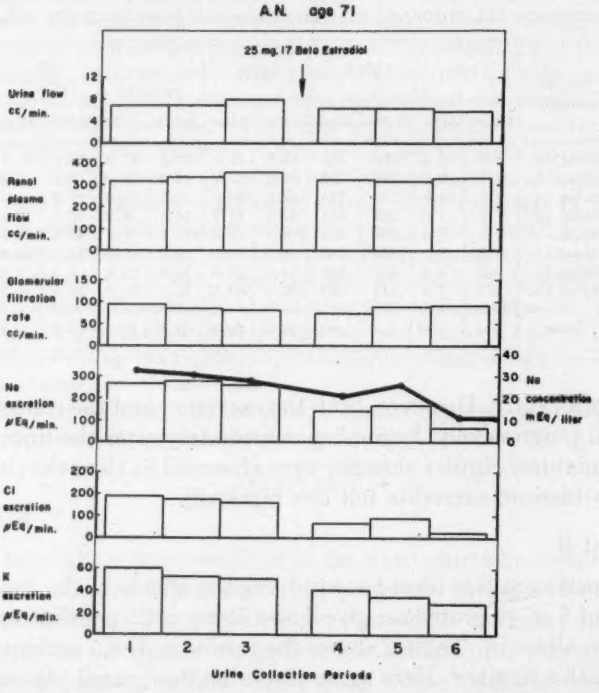

FIG. 1. Urinary data in a patient who received an intravenous injection of 25 mg. of estradiol dissolved in 5 cc. of propylene glycol and mixed with 50 cc. of 25 per cent salt-poor human serum albumin. Note the insignificant changes in urine flow, renal plasma flow and glomerular filtration rate. Both the excretion (blocks) and the concentration (running line) of Na fell sharply after the injection of the mixture. The excretion of Cl followed a similar trend. The excretion of K fell less than that of Cl and Na.

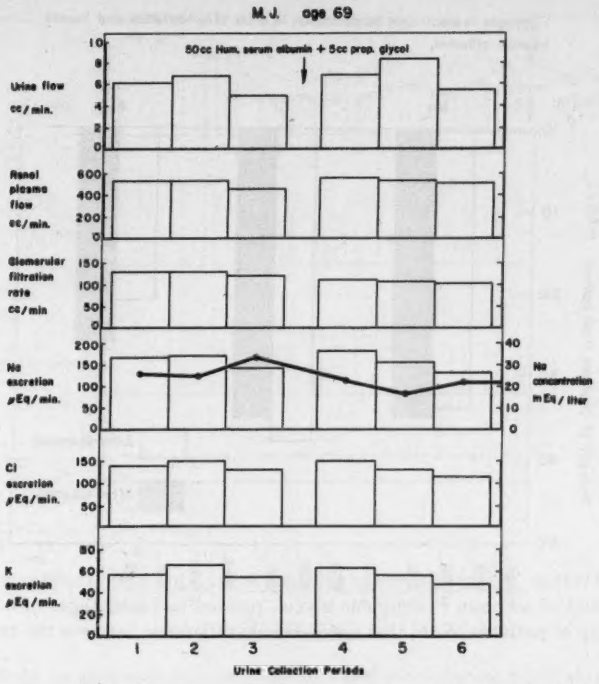

FIG. 2. Urinary data in a patient who received an intravenous injection of the vehicle alone—50 cc. of human serum albumin and 5 cc. of propylene glycol. This patient showed the least response to the injection. Nevertheless, the changes were not significantly different from those observed after the injection of the hormone mixture.

hemodynamics and excretion of electrolytes did not differ from that observed following administration of the hormone mixture.

Figure 3 shows a comparison of the average percentile changes in electrolyte concentration in the group of patients who received human serum albumin and hormone with the same values when the hormone was omitted from the mixture. It is clear that there was no significant difference between the two groups. The effects of intravenous estradiol on the vaginal smear were studied in 5 patients. Repeated smears were examined before and after the injection (*see* Material and Methods). No significant changes were observed in the vaginal smear up to six days after the injection of the hormone.

Experiment C

In view of the failure to detect any effect of intravenously administered estradiol, it was decided to study the effects of daily intramuscular injec-

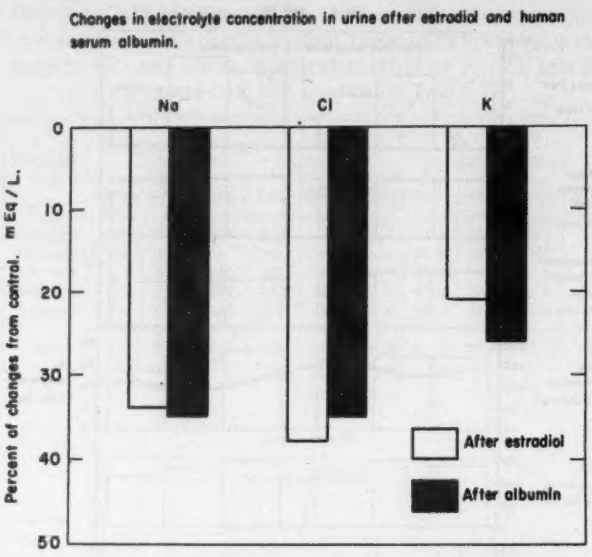

FIG. 3. Average changes in electrolyte concentrations after injections of albumin +estradiol and of albumin+propylene glycol, plotted as percentages of control values for each group of patients. Note that there was no difference between the two groups.

tions. Figure 4 shows data on 1 patient who was selected as representative of the whole group. The excretion of sodium and chloride during the four days of the control period averaged 105 mEq. and 96 mEq. per day, re-respectively. The intramuscular administration of estradiol resulted in a prompt fall in the excretion of both ions, which lasted for the entire treatment period. A slow rise in the excretion of sodium and chloride was observed during the post-treatment control period, but the excretion of these ions did not return to control values, even four to five days after cessation of therapy.

The decreased excretion of sodium and chloride during estrogen administration was mainly due to a fall in their concentration in the urine. However, a certain quantity of water was retained, probably isosmotically, with electrolytes as shown by the slight decrease in urine output and the increase in weight observed during hormone administration.

During the period of estradiol injection, the excretion of potassium showed a slight decrease, which persisted even after interruption of treatment. Despite the decrease in electrolyte excretion induced by estradiol, the level of plasma electrolytes and the renal glomerular filtration rate did not change.

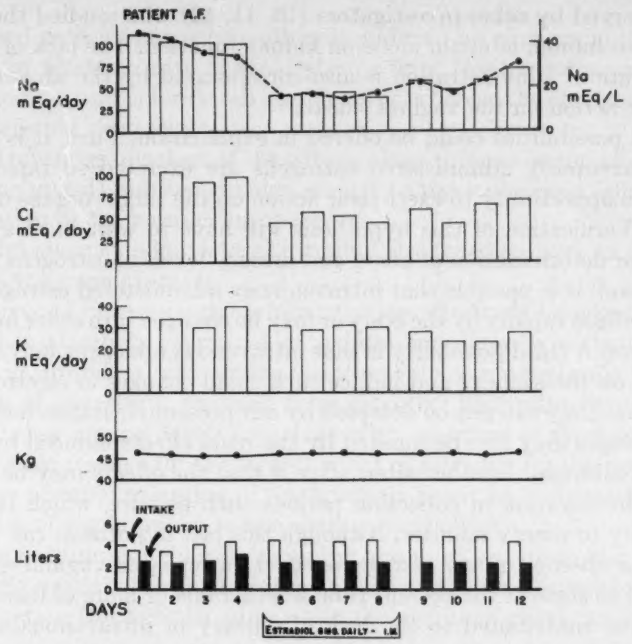

FIG. 4. Daily balance data of a patientwho was studied during a four-day control period, a four-day period of treatment with intramuscular estradiol, and a four-day recovery period. Note the prompt fall in both urinary exretion (blocks) and concentration (interrupted running line) of Na. Urinary Cl followed a similar pattern. The changes in K were not as striking as those in Na and Cl. Urinary output decreased slightly during hormone administration. All of these values had not returned to control levels even four days after cessation of therapy. It is probable that slow absorption of the hormone accounts for the prolonged action.

DISCUSSION

Intravenous administration of sex steroid hormones has been used recently in the treatment of a variety of endocrine disorders. Some authors (8, 9) have claimed, on the basis of clinical observations, that such a route offers advantages when rapid action is desired. However, there seems to be no concrete experimental evidence that intravenous injection has characteristic effects on the target organs. The present data show clearly that intravenous administration of doses up to 50 mg. of estradiol (equivalent to several times the daily output of the ovaries) did not elicit a more profound effect upon electrolyte excretion than did the injection of the vehicle alone. In fact, the renal effects observed in the present studies are very similar to

those observed by other investigators (10, 11, 12) who studied the effects of salt-poor human albumin alone on kidney function. The lack of efficacy of this route of administration is also corroborated by the absence of an estrogenic action on the vaginal smear.

Several possibilities could be offered in explanation. First, it is possible that intravenously administered estrogens are excreted so rapidly that there is no opportunity to exert their action on the target organs or on the kidneys. Verification of this hypothesis will have to wait until a reliable method for determination of blood and urinary levels of estrogens is available. Second, it is possible that intravenously administered estrogens may be metabolized rapidly by the body or may be changed into other nonactive metabolites. A third possibility is that intravenous estrogens may act very fleetingly on the kidneys and induce such small changes in electrolyte excretion that they can not be detected by our present clearance techniques. These changes may also be masked by the renal effects induced by the injection of albumin. Another alternative is that the effects may be delayed beyond the duration of collection periods such as ours, which lasted for about sixty to ninety minutes. Although this last hypothesis can be ruled out by the absence of any estrogenic effect on repeated vaginal smears, it is difficult to state at the present time whether one or more of these factors might have contributed to the lack of efficacy of intravenous estradiol.

In contrast to the absence of effects by the intravenous route, definite sodium and chloride retention was evoked by intramuscular injections of estradiol given over a period of a few days. The effects seemed to be more selective toward these ions, since potassium excretion was not greatly altered. Furthermore, with the possible exception of the amount of water which was reabsorbed isosmotically with electrolytes, estrogens did not seem to have any specific action on water excretion. Since there were no changes in either the plasma electrolyte level or in the renal glomerular filtration rate during the hormonal treatment, it may be assumed that the filtered load of electrolytes was not affected by estrogens and that the decreased excretion was mainly due to an increase in tubular reabsorption of these ions. Whether estrogens act directly on the renal tubules or indirectly through the adrenals or any other neuro-humoral mediator is the object of further investigations in these laboratories.

SUMMARY AND CONCLUSIONS

1. Effects of acute and chronic administration of estradiol on renal hemodynamics and electrolyte excretion were studied in 18 postmenopausal or surgically castrated women.

2. Intravenous administration of estradiol dissolved in propylene glycol

and mixed with human serum albumin induced no changes in urine flow, glomerular filtration rate or renal plasma flow. Concentration and excretion of sodium and chloride fell significantly, whereas the changes in potassium excretion were slight.

3. Intravenous injection of the vehicle alone (human serum albumin and propylene glycol) induced changes similar to those observed following administration of the hormone mixture.

4. Intravenously administered estradiol also failed to exert an estrogenic effect on the vaginal smear.

5. Repeated intramuscular administration of estradiol evoked a significant fall in the daily excretions of sodium and chloride without parallel changes in urine flow, glomerular filtration rate or renal plasma flow. The excretion of potassium changed inconsistently. Electrolyte excretion remained below control levels four days after cessation of therapy.

6. Possible reasons for the lack of effect of estradiol by the intravenous route are: a) rapid excretion of the hormone from the body, and b) rapid conversion of the hormone to non-active metabolites.

7. The sodium and chloride retaining action of intramuscular estradiol is probably mediated through a direct action on the renal tubules, although indirect action through the adrenals or the pituitary gland can not be excluded.

Acknowledgments

The authors are indebted to Dr. Robert Gaunt of Ciba Pharmaceutical Products, Summit, New Jersey for the supply of crystalline estradiol; to Cutter Laboratories for the supply of human serum albumin; and to Dr. E. Margolis of E. S. Miller Laboratories for the supply of estradiol dissolved in butylene glycol.

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SYNDROME OF STEATORRHEA, PSEUDOHYPOPARATHYROIDISM AND AMENORRHEA

OBSERVATIONS ON THE CALCIUM INFUSION TEST, AND THE EFFECT OF PROBENECID ON CALCIUM AND PHOSPHORUS METABOLISM

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STEATORRHEA usually results in a deficient absorption of phosphorus as well as calcium, and thus may lead to a lowered serum inorganic phosphate level, and even to osteomalacia. The finding of an elevated serum phosphate level in a case of steatorrhea with tetany was, therefore, unexpected, though not unique. It led, however to certain detailed observations and to the attempted delineation of a "new" syndrome.

CASE HISTORY

M.P. (No. 332532), a 32-year-old intelligent, unmarried, colored female domestic servant had suffered from diarrhea for two months. This consisted of five stools daily, which were pale, bulky and of unpleasant odor. Four days before admission the diarrhea subsided, but tetany developed, which brought her to hospital on July 28, 1954. She had lost 7 pounds during this period. Menses up till this time had been normal and regular. Examination revealed a lean young woman, who did not appear ill. She weighed 112 pounds, and exhibited carpal spasms, a Chvostek sign and mildly clubbed fingers. Her urine was normal when tested on the ward. The hemoglobin level was low (10 Gm. per cent, with a packed cell volume of 39 per cent) and the red cells showed hypochromia. The serum calcium level was 6.6 mg. per 100 ml. Calcium gluconate was injected intravenously during her first three days in the hospital, in amounts just sufficient to control the tetany; it was then discontinued. She has had three hospital admissions. During her first two-month hospital stay and since that time, she has had several relapses, when steatorrheic stools are passed for a week or so, and tetanic symptoms recur. Interspersed are longer remissions, during which she feels perfectly well and strong; her stools, though pale, are passed only once daily, and even a Chvostek sign is difficult to obtain. During the past year her finger clubbing has become more pronounced, her bones have become definitely decalcified, her periods have diminished and are now absent, and, at

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one time, her hemoglobin level was as low as 7 Gm. per 100 ml. The anemia has remained hypochromic in type. She has continued at work, except for her three spells in the hospital, and insists that for the most part she is perfectly well. She has been afebrile throughout, and her erythrocyte sedimentation rate has not been raised. She has lost no more than 3 or 4 pounds since her first admission.

Initial laboratory investigations (*see* Appendix for methods): The concentration of blood urea was 27 mg. per 100 ml. The level of serum sodium was 148 mEq./L, potassium 3.4 mEq./L, chloride 100 mEq./L, CO_2 combining power 59 vol. per 100 ml., inorganic phosphate 4.1–6.7 mg. per 100 ml. (over 50 estimations with a mean value around 5.4 mg.), and calcium 6.6–9.5 mg. per 100 ml. (being low during relapses and bearing no apparent relationship to the level of serum phosphorus). The blood Wassermann reaction was negative. The prothrombin index was 91, thymol turbidity 1, and thymol flocculation 0. The level of serum albumin was 3.3–4.3 Gm. per cent, globulin 1.9–2.2 Gm. per cent, and alkaline phosphatase 7–9 Bodansky-Shinowara units. A fractional test meal showed achlorhydria even after histamine (two occasions).

X-ray examination revealed a normal chest. There was some demineralization of the skeleton but no definite evidence of osteomalacia (a year later the decalcification was more evident). A straight roentgenogram of the abdomen showed multiple dilated jejunal loops containing fluid. There was no calcification in the pancreas. After a barium meal (using barium sulphate suspension) the abnormal jejunal pattern was confirmed, with clumping and moulage. There was no evidence of other disease.

Examination of the stomach contents and stool for blood, tubercle bacilli and parasites yielded negative results. Reaction to the Mantoux test was weakly positive at 1/1000. The glucose tolerance curve was flat and represented the following blood sugar values: fasting 94 mg.; (50 mg. of glucose given orally); one-half hour 112 mg.; one hour 119 mg.; one and a half hours 112 mg.; two hours 114 mg.; and two and a half hours 103 mg. per 100 ml. The bone marrow was normal. The serum concentration of vitamin B_{12} was 100 μg. per 100 ml. (a low-normal value in our laboratory—Dr. D. Mackenzie). A three-day fat balance study (intake 70 Gm. per day) showed 75 per cent and 66 per cent utilization during relapse, and 92 per cent utilization during remission. The glomerular filtration rate (inulin clearance) was 150–220 ml. per minute.

FURTHER CONSIDERATIONS

A. *Pancreatic function*

But for the high serum phosphorus level, the foregoing description seemed fairly conclusive for the diagnosis of "idiopathic steatorrhea" (non-tropical sprue). However, a duodenal intubation, performed under fluoroscopic control, showed only a trace of trypsin in several samples of aspirated juice, even after stimulation by 20 per cent cream. There was also a gross deficiency of amylase content (20–1,000 Wohlgemuth units in different samples). Several samples of steatorrheic stool likewise showed no tryptic activity.

Despite this unexpected lack of pancreatic enzymes, the balance of evidence remained strongly against a *primary* pancreatic cause of the steatorrhea. The clubbed fingers, anemia, presence of tetany, small intestinal pattern and flat glucose tolerance curve all favored the idiopathic form of steat-

orrhea (1). A deficiency of pancreatic enzymes may occasionally occur in a grossly undernourished adult (2), but M.P. was never so severely ill.

B. *Sexual functions and the pituitary*

After the first hospital admission, amenorrhea developed (scanty periods at first, and complete absence later). A vaginal examination in July 1954 revealed nothing abnormal, yet in April 1955 the cervix was reported as being atrophic, the uterus hypoplastic and the adnexae not palpable. Endometrial biopsy showed very atrophic endometrium. Two determinations of urinary follicle-stimulating-hormone were made, and each time the result was negative at 6 mouse units. It seems plain, therefore, that the hypogonadism which had developed was secondary to lack of pituitary gonadotropin. Other tests of pituitary function yielded normal results. The basal metabolic rate was +12 per cent, and insulin tolerance and hypoglycemia responsiveness were normal. The patient had never been pregnant. She did not seem ill enough to justify considering the pituitary depression to be a nonspecific effect of severe emaciating disease or undernutrition. She showed no stigmata of acromegaly (which might have accounted for the high serum phosphorus level, the supernormal glomerular filtration rate and the amenorrhea with low FSH), and the sella turcica was not enlarged.

C. *High serum phosphorus level and parathyroid function*

An elevated serum inorganic phosphate concentration in a case of steatorrhea was a most unexpected finding since, in our experience, the serum phosphorus level tends uniformly to be low, especially when there is decalcification of bone. In 1932 Bennett, Hunter and Vaughan (3) describing the syndrome of "idiopathic steatorrhoea," remarked that 5 of their 15 cases showed serum phosphorus readings above 5 mg. per 100 ml.; in 1 case the reading was 6.3 mg. They offered no explanation, beyond the trite remark that secondary hypoparathyroidism may have been present. At first glance, this would seem unlikely in association with a low serum calcium level, which is believed actually to stimulate parathyroid activity. The tendency to a low serum calcium level in steatorrhea would therefore be expected to induce secondary hyperparathyroidism rather than hypoparathyroidism (4).

In view of the anomalous finding of an elevated serum phosphorus concentration in our patient, more complete biochemical studies were undertaken.

The urinary phosphorus output was found to be remarkably low, notwithstanding the high serum level. It varied between 200–400 mg. daily on an intake of about 1 gram. Since renal glomerular function was excellent, the low excretion suggested phosphorus conservation by increased tubular

reabsorption. This is typical of hypoparathyroidism, as one action of parathyroid extract is to maintain the urinary phosphorus by diminishing tubular reabsorption (4).

The serum calcium concentration, though generally low, was at times well above 9 mg. per 100 ml. The urinary calcium output remained very low (30 mg. or less in twenty-four hours), and did not increase in association with higher serum levels.

Response to parathyroid hormone. The association of a high level of serum phosphorus with a low level of serum calcium and low urinary excretions of calcium and phosphorus constitutes a combination characteristic of hypoparathyroidism. The response to parathyroid extract was therefore investigated (5). The patient showed no significant phosphate diuresis.

The same batch of parathyroid extract (Parathormone, Lilly) caused an excellent phosphorus diuresis (threefold increase) in a normal subject, and in a known postoperative hypoparathyroid subject (H.W., Fig. 1). Since it has been claimed that enormous changes in the phosphorus output may occur from hour to hour during the course of a day, the normal control subject was tested both morning and afternoon, to make sure that our Parathormone was really active. Each time a satisfactory phosphate diuresis

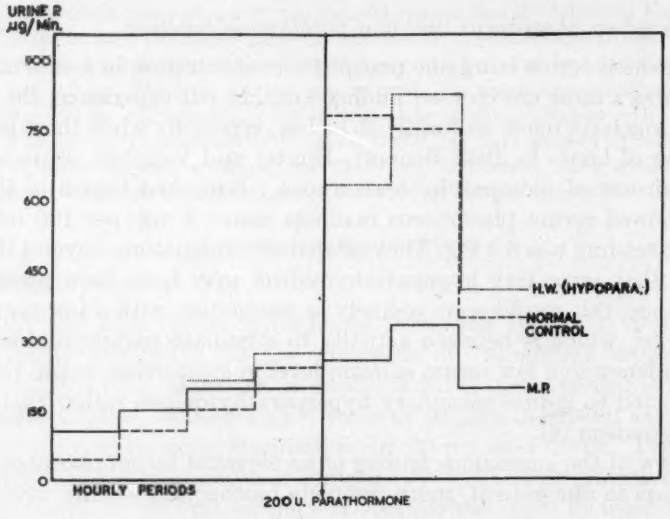

FIG. 1. Lack of response to parathyroid extract (Parathormone) in Patient M.P. compared with Patient H.W. (known postoperative hypoparathyroidism) and a normal subject.

was obtained. We concluded that M.P. was insensitive to parathyroid extract.

Comment. It is plain, therefore, that M.P. did not have primary hypoparathyroidism; in fact her renal tubules were not even normally sensitive to parathyroid extract. However, she did not show the specific physical characteristics of pseudohypoparathyroidism (6). Recently, it has been realized that there is less absolute distinction between the two types of hypoparathyroidism than was previously thought (7). Thus the resistance to parathyroid hormone in pseudohypoparathyroidism is relative rather than complete. Moreover, very resistant cases have been reported in which the physical characteristics of the typical pseudo-syndrome are lacking (8).

To summarize, it would appear that M.P. had "idiopathic steatorrhea," unexplained failure of pancreatic enzyme production, and the biochemical picture of hypoparathyroidism with inability of her renal tubules to respond to parathyroid extract. Lowe *et al.* (9) reported remarkably similar findings in a young girl who was observed from birth to have tetany and a high serum phosphorus level (up to 6.2 and 7 mg. per 100 ml.). The steatorrhea was associated with fat absorption of only 65 per cent, a hemoglobin level of 9.7 Gm. and an increased prothrombin time. Pancreatic enzyme production was apparently depressed, but the aspirated duodenal juice was normal when the test was repeated at a later date. There was no response to administration of an active Parathormone preparation. Like M.P., therefore, the patient of Lowe *et al.* had "idiopathic steatorrhea" with a high concentration of serum phosphorus, possible initial pancreatic anenzymia, and resistance to parathyroid hormone. The authors suggest that the low serum calcium level led to continued excessive parathyroid hormone production, which caused constant stimulation of the renal tubules and led to their eventually becoming refractory; thus urinary phosphorus excretion was reduced and the serum level increased. They adduce no evidence for this hypothesis, and although the possibility of a refractory state in their very chronic case might be admitted, this seems to be most unlikely in M.P., whose symptoms were of such short duration. However, since about 20 per cent of patients with idiopathic steatorrhea have no bowel complaints (1), it is possible that biochemical aberrations existed in M.P. for a much longer time than her story suggests.

Another interesting patient showing the association of hypoparathyroidism and steatorrhea was discussed in the case records of the Massachusetts General Hospital (10). In this patient, the steatorrhea appears to have been mild, and is mentioned only as an incidental finding. The response to parathyroid extract is not discussed, but at autopsy no recognizable parathyroid was found.

D. *Calcium metabolism and response to vitamin* D

The urinary calcium output remained exceedingly low, even during periods of remission when normal serum calcium values were obtained. This is reminiscent of some cases of rickets due to lack of vitamin D, in which the serum calcium level is within the normal range. If we assume that no gross changes in the diffusibility of the calcium occur and that good glomerular function is present, the only explanation is an excessive tubular reabsorption of filtered calcium. In rickets, vitamin D may restore the level of urinary calcium to normal before restoring the level of serum calcium. This plainly suggests that vitamin D itself may have a direct calciuric effect. A similar phenomenon may be seen in hypoparathyroidism under treatment with vitamin D—the urinary calcium in some cases may be excessive, while the serum calcium level is still low. In M.P., however, 50,000 units of vitamin D given parenterally daily did not affect the levels of urinary or serum calcium or phosphorus; in fact, the tetany actually became worse.

E. *Response to probenecid (Benemid®)*

In true hypoparathyroidism, probenecid is said to induce a phosphorus diuresis (11, 12). This is not seen in normal subjects, in whom the regulatory activity of parathyroid hormone would appear to mask such an action. In M.P., administration of Benemid for eight days[1] had no effect on the urinary phosphorus, but the urinary calcium rose from around 30 mg. a day to about 80 mg. (Fig. 2, upper chart).

The serum phosphorus level fell distinctly, despite the lack of phosphate diuresis. This dissociation of effect on serum and urinary phosphorus was not found in 1 case of pseudohypoparathyroidism reported by Pascale *et al.* (11). These workers were able to attribute the drop in serum phosphorus concentration in their cases to the increased urinary excretion. In M.P., on the other hand, it is plain that the fall in serum phosphorus concentration cannot be explained by loss of phosphorus in the urine. Similar results were obtained when a known postoperative hypoparathyroid subject (H.W., Fig. 2, lower chart) was given Benemid in a dosage of 1 Gm. every six hours. Contrary to reports in the literature, there was no change in urinary phosphorus, but the serum phosphorus level dropped from 4.7 to 3.7 mg. per 100 ml. The urinary calcium increased, but there was no change in the serum calcium level nor in the Chvostek and Trousseau signs. This experiment was repeated and the same results were obtained. In this patient also we thus found a dissociation of effect on serum and urinary phos-

[1] Doses were 0.5 Gm. every six hours for four days; then 1.0 Gm. every six hours for four more days.

phorus. The probenecid must have induced the alteration in serum phosphorus concentration by reduction in fecal phosphorus or by redistribution of phosphorus from the intracellular compartment.

A calciuric effect of probenecid is not a well-documented finding, though Pascale *et al.* (11) record an almost parallel rise in calcium excretion in 1 of their hypoparathyroid patients treated with this substance. A similar rise is to be found in their charts of the only other case in which the urinary calcium was recorded.

In a pilot survey we found no calciuric effect of probenecid in normal or in gouty subjects, but in a patient with Kinnear-Wilson's disease and in one with sarcoidosis, the rise in urinary calcium was considerable.

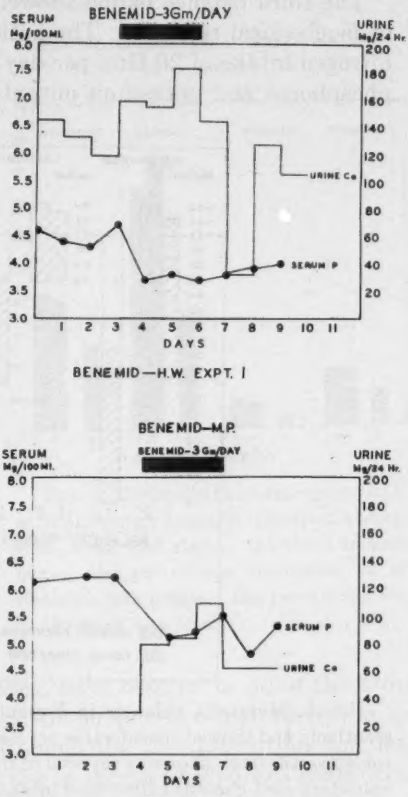

FIG. 2. Response of Patient M.P. (upper chart) and of a postoperative hypoparathyroid subject (H.W., lower chart) to probenecid (Benemid). The serum calcium level, and the urinary phosphorus were not altered, and are not charted.

F. *Metabolic balance studies*

Three five-day metabolic balance studies were performed while the subject was receiving a normal ward diet. The first two studies were run consecutively and the third some months later. The food, urine and stools were analyzed for calcium, phosphorus, nitrogen and potassium content. Results are shown in Figure 3, charted after the manner of Reifenstein, Albright and Wells (13). The diet had remained unchanged for at least two weeks before each balance period.

During the first two periods, M.P. showed a marked negative balance for all elements measured. The fecal calcium was actually higher than the intake.[2] There was more phosphorus in the stool than in the urine. The fecal potassium loss was high (27 mEq. per day)—reminiscent of pa-

[2] This could occur if food such as milk was drunk in excess of that provided in the diet; but, as far as we could tell, this was not the case.

tients with more chronic steatorrhea in whom hypopotassemia develops. The fecal nitrogen was also high (nearly 20 per cent of intake). The over-all nitrogen loss was equivalent to about 1 ounce of protein per day.

The third balance period showed the dramatic change during a period of biochemical remission. The patient had a ravenous appetite (note the nitrogen intake of 20 Gm. per day). Although there was still a high fecal phosphorus and potassium output, there was a strong positive balance.

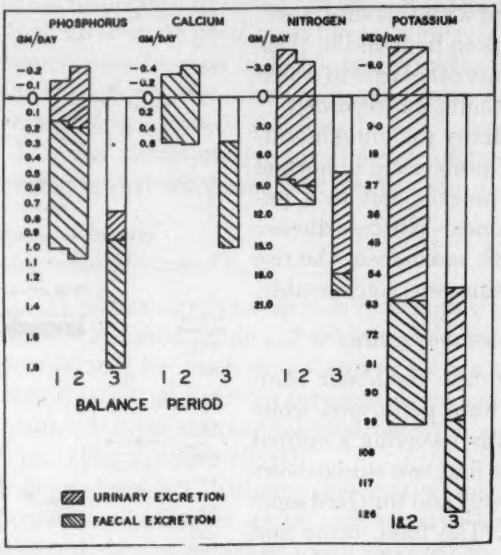

FIG. 3. Metabolic balances in Patient M.P. The first two 5-day periods ran consecutively and showed considerable net loss of all four elements. The third balance study, some months later, showed a reversal of this trend during a period of remission. Note the voluntary (self-chosen) higher food intake.

Figure 4 shows the fall in fecal excretion of all four elements, expressed as a percentage of the intake, and indicates a very considerable rise in absorptive capacity. These values demonstrate the extraordinarily intermittent character of M.P.'s illness, and explain also why she did not lose weight during the past year.

G. *Trial of gluten-free diet*

Calcium chloride by mouth was found to be successful in relieving the tetany and partially raising the serum calcium level. In view of the good results with gluten-free diet in celiac disease (14, 15) and its occasional good

effect in adult steatorrhea (16), it was tried as definitive treatment in M.P. Success appeared to be almost immediate, with diminished frequency of stools, disappearance of tetany and an increase in the serum calcium level. A two-day relapse was traced to the eating of an ice-cream cone made from wheat flour when she was allowed out one Sunday. However, there was no relapse after discontinuing this line of therapy and it is evident that the remission was merely coincidental.

We had hoped to try hydrocortisone, which may be very effective in idiopathic steatorrhea (17, 18), but unfortunately M.P. became hospital-shy.

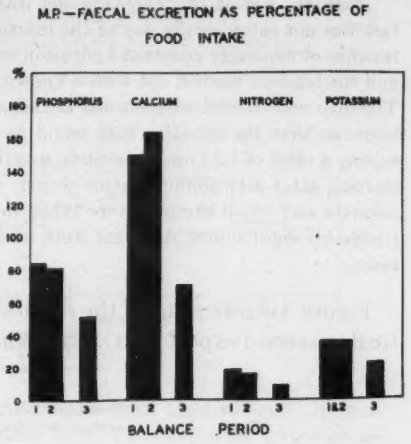

FIG. 4. Metabolic balances expressed so as to indicate change in intestinal absorption. Note that during the third balance period the percentage absorption of all elements was greater; the percentage loss in the stool was less.

CALCIUM INFUSION EXPERIMENTS[3]

It has recently been reported (19–22) that the rapid intravenous infusion of calcium gluconate into normal subjects will cause a fall in the urinary phosphorus and a rise in serum phosphorus concentration, supposedly due to a depression of parathyroid gland activity by the rising serum calcium level. Not all normal subjects, however, have reacted in quite the same way; Kyle *et al.* (21) reported on 1 control subject in whom there was an initial rise in urinary phosphorus, although the 24-hour output was less than that of two previous days. The rise in serum phosphorus concentration in the normal subject is far greater than can be explained by the conservation of phosphorus resulting from diminished phosphaturia; nor can it apparently be explained by a diminution in fecal excretion of phosphorus, since Baylor found no change in this factor (19). It would appear, then, that the rapid rise in the level of serum calcium itself, in some way mobilizes phosphorus into the blood stream (and presumably into the extracellular fluid as a whole), by a redistribution from the intracellular compartment. In hypoparathyroidism, a marked increase in urinary phos-

[3] *Note added in proof:* Further analysis of the calcium infusion results and investigations on other subjects (with Dr. E. B. Dowdle) indicate that the discussion in this section is incomplete and probably oversimplified. We hope to publish our results shortly.

phorus is usually found following calcium infusion (20–23). Again the serum phosphorus level rises. The mechanism is unexplained.

The calcium infusion test was performed twice on M.P. and also on 2 healthy control subjects (Figs. 5 and 6).

Methods. A relatively constant diet was taken for several days before the test. Breakfast was not eaten on the day of the infusion, which was started about 9 A.M. The maintenance of relatively constant hydration was attempted. An indwelling catheter was used and the bladder washed out with a known volume of distilled water at each evacuation. The drip was started with normal saline, at about 15 drops a minute. Inulin was added later, so that the filtration rate could be assessed throughout. Calcium gluconate, to supply a total of 1.3 Gm. of calcium, was infused over a period of from one to two hours, starting after a two-hour control period. Urine was withdrawn every twenty or thirty minutes and blood samples were taken midway between. The saline infusion was continued for eight hours. Analyses were made for calcium, phosphorus, inulin and creatinine.

Figure 5 shows some of the results in M.P. (the findings were almost identical in second experiment). By taking more frequent blood and urine meas-

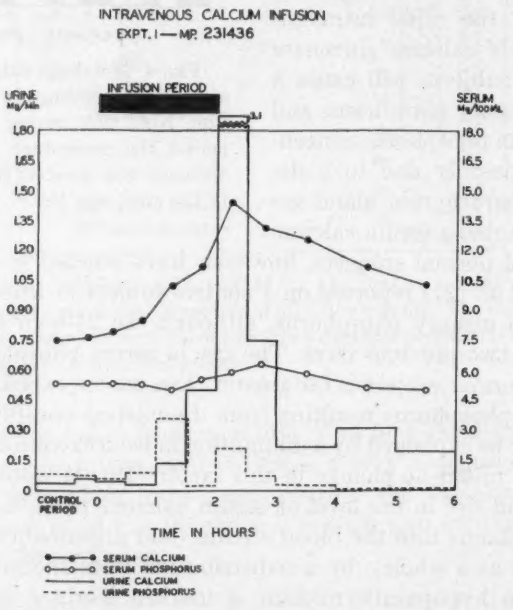

FIG 5. Results of intravenous infusion of 13.5 Gm. of calcium gluconate in Patient M.P. Note, during the control period, the high serum phosphorus level, low urinary phosphorus, low serum calcium level, and low urinary calcium; the increase in urinary phosphorus during calcium infusion; the later rise in serum phosphorus concentration, and finally the decrease to virtual absence of phosphorus in the urine.

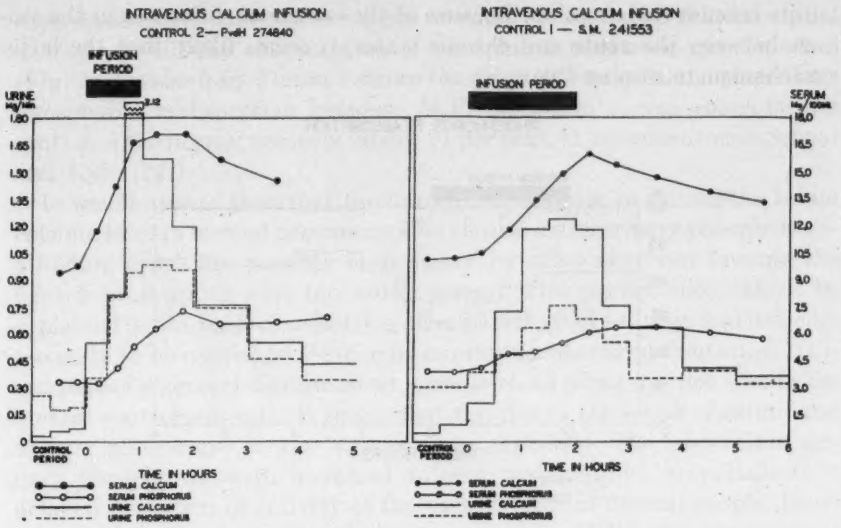

FIG. 6. Similar calcium infusions in 2 young normal control male subjects. In general, the response was the same as that in Patient M.P. The rise in the serum phosphorus level was even more marked. Note in all cases the lack of temporal relationship between changes in the levels of serum and the urinary phosphorus. In these controls the urinary phosphorus excretion did not fall below the pre-infusion level by the end of five or six hours.

urements than have been reported elsewhere, the early changes consequent upon acute raising of the serum calcium level were observed, together with their time relations to each other. Thus, it was found that:

1) There was a very rapid rise in urinary phosphorus, followed within two or three hours by a depression amounting to a virtual absence of phosphorus from the urine. This (Fig. 7) represents an initial lowering of tubular reabsorption of phosphorus followed by a rise to virtually 100 per cent.

2) There was a more delayed rise in the serum phosphorus level, with its peak well after that of the urinary phosphorus. It plainly could not have *caused* the increased phosphaturia.

3) At a serum calcium level of 9.4 mg. per 100 ml. the urinary output was 0.8 mg. per minute (figures from the second experiment). This rate of excretion would total a daily calcium output of about 1,100 mg; yet, when M.P. had a normal serum calcium level of 9 mg. per 100 ml. her daily output was less than 30 mg. It seems that acute changes in serum calcium concentration may cause phenomena different from those following more chronic changes (23). Some sort of adaptation must occur over longer periods—either a reduction in the filterability of the calcium or an increase

in its tubular reabsorption. Because of the enormous difference in the values between the acute and chronic states, it seems likely that the latter mechanism must play a part.

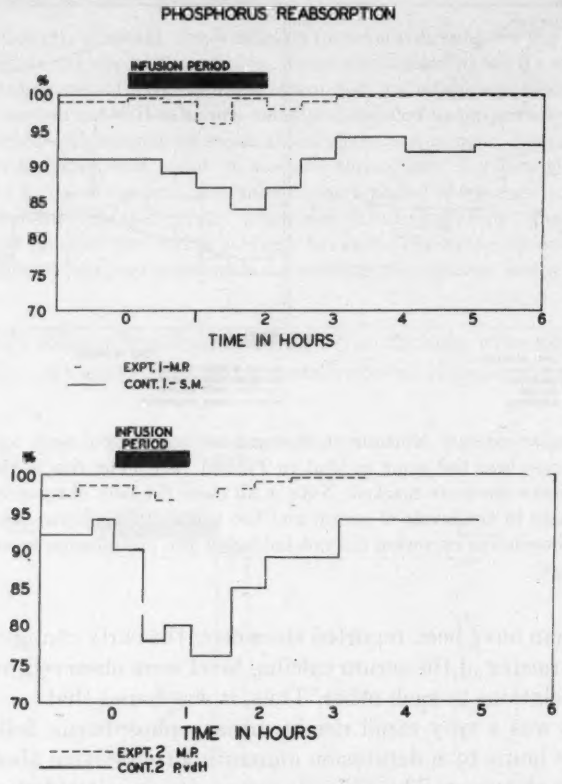

FIG. 7. Phosphorus reabsorption by the renal tubules in Patient M.P. and in normal control subjects. Note the fall in reabsorption caused by calcium gluconate infusion. The much higher reabsorption in Patient M.P. is characteristic of hypoparathyroidism. Reabsorption here is expressed as a percentage of the filtered load.

4) The glomerular filtration rate increased slightly during the infusion to a maximum of 230 ml. per minute. The minute volume increased remarkably to a maximum of 16 ml. per minute.

An initial rise in urinary phosphate has also been reported in cases of known hypoparathyroidism, so that this finding in M.P. appeared to support our suggested diagnosis. However, 2 normal control subjects were also tested and to our surprise showed the same sort of results (Fig. 6) with regard to phosphorus and calcium. Furthermore, in their tests there was no

significant change in minute volume or inulin clearance, so that the initial decrease in tubular reabsorption of phosphorus was even more marked (Fig. 7). Incidentally Figure 7 shows the great difference in the percentage phosphorus reabsorption between M.P. (in whom it was about 99 per cent) and the normal subjects (about 91 per cent, in agreement with Schaaf and Kyle (24)).

It would appear then that the immediate response to raising the serum calcium level in normal persons may be an *increase* in urinary phosphorus—a finding which has possibly been missed by other observers because the periods of sampling were too widely spread. This phenomenon cannot be explained on the basis of a shutting off of parathyroid activity, and it occurs too early to be related to the rise in serum phosphorus concentration. It is not part of a general diuretic effect, since such an effect was not seen in the normal control subjects. It seems that the rise in the levels of serum and urinary phosphorus cannot at present be explained. The later fall in urinary phosphorus, with increased tubular reabsorption, may indicate a delayed inhibition of activity of the parathyroids in normal people. However, this can hardly explain the same response in M.P., who was resistant to parathyroid extract, but whose tubular reabsorption of phosphorus actually approached 100 per cent after the initial fall.

It is interesting that the changes in the level of serum phosphorus with both probenecid and intravenous calcium cannot be explained on the basis of excessive loss or retention by the kidneys. A similar finding has been recorded in a case of pseudohypoparathyroidism in which parathyroid extract induced a rise in the serum phosphorus level, but no change in the urinary output of the element (25). It is suggestive, therefore, that the body may respond to several agents by a redistribution of phosphorus between extra-cellular and intra-cellular compartments, rather than by renal regulation.

SUMMARY

The case of a young woman presenting with tetany is discussed. She apparently suffered from "idiopathic steatorrhea" (non-tropical sprue), yet she had a deficiency in pancreatic enzyme production. There was a high level of serum inorganic phosphate, low urinary phosphorus and lack of response to administration of parathyroid hormone. She exhibited a type of "pseudohypoparathyroidism" with over-reabsorption of phosphorus by the renal tubules, apparently due to end-organ failure to respond to the parathyroid hormone. Amenorrhea also developed, in association with low urinary excretion of follicle-stimulating hormone, and uterine atrophy. Metabolic balance studies demonstrated the remarkable fluctuations in the course of her illness.

A few somewhat comparable cases are on record, particularly that of

Lowe and associates. We have described another example of a little recognized syndrome which might be found more often if attention were paid to the serum phosphorus level in all cases of steatorrhea.

In the investigation of this case, intravenous calcium infusion studies were performed. A marked diuresis occurred during the infusion and the glomerular filtration rate was discovered to be unusually high. There was an early rise in urinary phosphorus output followed by a later fall, and a rise in serum phosphorus level. Two normal control subjects reacted in the same way with regard to phosphorus metabolism and it is suggested that the earliest effect, even in the normal subject, may be a rise in urinary phosphorus, rather than a fall as is generally believed. Such an effect cannot readily be explained by invoking parathyroid responses.

The effect of probenecid on phosphorus and calcium metabolism was investigated in this case, and also in a patient with postoperative hypoparathyroidism. In each instance probenecid caused a fall in the serum phosphorus level without a change in urinary phosphorus, and a rise in urinary calcium without a change in the serum calcium level.

Acknowledgments

Our grateful thanks are due to Prof. F. Forman and Prof. J. Brock for constant interest and advice; to Drs. J. de Villiers and W. H. Muller for the gynecologic examinations; to Prof. H. Zwarenstein and Mr. D. G. Duncan for the FSH tests; to our technicians, to the dietitians at Groote Schuur Hospital, to the photographer, Mr. B. Todt, and to the University of Cape Town for a grant from its Staff Research Fund.

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APPENDIX

1. Chemical methods used:
   Serum calcium: Greenblatt and Hartman (26).
   Serum and urinary inorganic phosphate: King (27).
   Urinary calcium: Fiske and Logan (28).
   Nitrogen: Micro-Kjeldahl.
   Fecal fat: van de Kamer *et al.* (29).
   Inulin: Roe *et al.* (30).
   Sodium and potassium: Flame photometer.
   Trypsin: Northrop and Kunitz (31).
   Amylase: Wohlgemuth (32).
   Urinary FSH: Bloomberg *et al.* (33).
   Glucose tolerance: Capillary blood, Hagedorn-Jensen method.
   Metabolic balance: Reifenstein, Albright and Wells (13).
2. The results of balance studies, and Benemid and calcium infusion experiments have been expressed in charts, rather than in tables listing exact figures, in order to conserve space. The relevant tables may be obtained from the authors on request.

COMPARATIVE EFFECTS OF PREDNISONE AND OF CORTISONE IN SUPPRESSING THE RESPONSE OF THE ADRENAL CORTEX TO EXOGENOUS ADRENOCORTICOTROPIN*

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ADMINISTRATION of adrenocortical steroids in a great variety of disease states is now so widespread that it has become essential for the clinician to have a detailed understanding of the physiologic and chemical changes brought about by these potent agents. One of the more important steroid effects—suppression of endogenous pituitary-adrenal function—has been recognized since Ingle's demonstration that long-term administration of adrenocortical extract results in atrophy of the adrenal cortex in animals (1). Sayers and Sayers (2) and others later showed that several of the adrenocortical steroids can inhibit the release of corticotropin in response to stressful stimuli, such as heat, cold and histamine. Adrenal extract does not interfere with the tropic action of administered corticotropin on the adrenal cortices of hypophysectomized animals (1, 3). Therefore, it has been assumed that the adrenocortical hormones cause adrenal atrophy in the intact animal by preventing the release of corticotropin from the adenohypophysis, and not by blocking the action of corticotropin on the adrenal cortex itself.

Clinical evidence of adrenocortical hypofunction was reported by Sprague and his co-workers, who found clinical symptoms and signs of depressed adrenal activity (asthenia, low urinary 17-ketosteroid values) following large doses of cortisone (4).

From these data, Sayers (3) postulated that the titer of circulating adrenocortical hormone is an important factor in regulating the rate of dis-

Received November 30, 1955.

* Aided by grants from the United States Public Health Service, the National Institute of Arthritis and Metabolic Diseases (A-195); the Upjohn Company; and Merck, Inc.

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charge of corticotropin from the anterior pituitary. Additional evidence in favor of this concept is the finding of increased blood levels of corticotropin in patients with Addison's disease, who have little or no circulating adrenal hormone (5).

Further clinical evidence in support of the idea that prolonged corticosteroid administration may cause adrenal hypofunction was provided by the observations of Fraser *et al.* (6a) and Salassa and his co-workers (6b). These authors described fatal postoperative adrenocortical insufficiency after cessation of long-term treatment with cortisone. Anatomical data bearing on this point were presented by Bennett (7), who reported adrenal atrophy and loss of lipid from cells of the adrenal cortex following cortisone administration; the pathologic changes, together with changes in the basophil cells of the anterior pituitary, persisted in some cases for several months after steroid withdrawal.

As a corollary to the postulate just cited, Sayers also theorized that the ability of a given steroid to inhibit activity of the pituitary-adrenal system parallels its anti-inflammatory potency (3). With the development of more powerful "anti-inflammatory" steroids than cortisone or hydrocortisone—for example, prednisone (8) (metacortandracin or delta-1-cortisone), it becomes important to learn whether these new compounds are also quantitatively more effective in causing adrenocortical suppression.

The purpose of this investigation was to compare the effect of prednisone with that of cortisone in depressing adrenal responsiveness to administered corticotropin. Plasma 17-hydroxycorticosteroid levels were used as indices of adrenal response. As may be seen in the section on Results, prednisone appears to be several times more potent than cortisone in suppressing the function of the pituitary-adrenal system. In addition, data were obtained concerning management of the adrenocortical hypo-responsiveness which occurs following withdrawal of steroids.

MATERIALS AND METHODS

The two steroids, prednisone[1] and cortisone, were administered orally to patients and to normal subjects according to various dosage schedules. The classification of patients with non-endocrine disorders as "normal" in terms of adrenocortical function seemed justified in view of the normal levels of plasma 17-OH-corticosteroid (9, 10) and the normal responses of these levels to corticotropin (11) observed in such subjects.

Adrenocortical response to corticotropin was tested before (whenever possible) and after administration of the steroid. The index of response, *i.e.*, rise in plasma 17-OH-corticosteroid levels after injection of corticotropin, has been described (12). In brief, the method consists of a four-hour intravenous infusion of 25 mg. of corticotropin

[1] We are indebted to Dr. Edward Henderson of Schering Corporation and to Dr. C. J. O'Donovan of the Upjohn Company for generous supplies of Meticorten® and Deltasone®, respectively.

(ACTH) dissolved in 500 ml. of 5 per cent dextrose and water. Plasma 17-OH-corticosteroid levels were determined according to the technic of Silber and Porter (13) on blood specimens obtained at the beginning and at the end of the standardized corticotropin test. Inclusion of exogenous corticosteroid in the values for plasma levels determined following administration of prednisone and cortisone was avoided by performing the post-steroid corticotropin tests twelve to twenty-four hours after the last dose of the hormone. Raile, Ely and Kelley have shown that the high plasma corticosteroid levels which result from oral cortisone decrease toward control values within eight hours (14).

RESULTS

Definition of normal adrenocortical activity

The effect of 4-hour intravenous administration of corticotropin on plasma 17-OH-corticosteroid levels in normal subjects and in patients without adrenocortical disease, not previously treated with adrenal steroids, is shown in Figure 1. In the patients presented subsequently, control (pre-steroid) plasma corticosteroid responses were within the normal limits defined in Figure 1 (12). Pre-corticotropin levels of plasma 17-OH-corticosteroids ranged from 4 to 23 micrograms per 100 cc. (within the normal limits of 4 to 28 micrograms per 100 cc. found in this laboratory). Post-

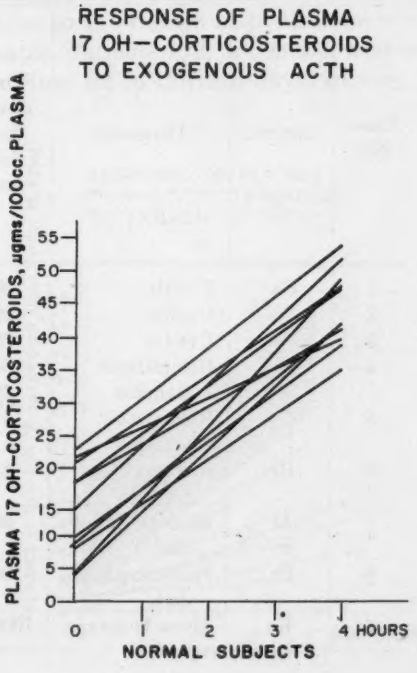

FIG. 1. The data presented in this graph were obtained from 11 normal subjects. (Results in 29 additional subjects without adrenocortical disease also fell within these limits.)

corticotropin levels were above normal, ranging from 35 to 55 micrograms per 100 cc. (12).

1. *Effect of prednisone in patients without adrenocortical disease*

Table 1 and Figure 2 show the results of standardized corticotropin (ACTH) tests in 9 patients who received prednisone. In 6 instances, the compound was given in small doses, 20–30 mg. daily, for short periods of from five to thirteen days (Patients 1–6, Table 1). In the remaining 3 (Patients 7–9, Table 1), dosage was at higher levels or the course was more prolonged. In 7 cases, pre-steroid corticotropin tests resulted in normal increases in plasma 17-OH-corticosteroid concentration, the responses falling within the shaded area indicated in Figure 2. The post-steroid responses, indicated by solid lines in Figure 2, were subnormal, resembling in slope the responses in patients with secondary adrenocortical insufficiency due to hypopituitarism (12). For comparison, Figure 3 is presented to show the "hypo-responsiveness" of plasma corticosteroid levels to

TABLE 1. EFFECT OF ORAL PREDNISONE THERAPY ON ADRENOCORTICAL RESPONSE TO THE STANDARD CORTICOTROPIN (ACTH) TEST IN ENDOCRINOLOGICALLY NORMAL INDIVIDUALS

Case No.	Patient	Diagnosis	Prednisone, daily dose (mg.)	Duration of therapy (days)	Plasma 17-OH corticosteroids (μg./100 cc.)			
					Before prednisone		After prednisone	
					Before ACTH test	After ACTH test	Before ACTH test	After ACTH test
1	Ca	Uveitis	20	7	31	49	8	19
2	Wi	Uveitis	20	7	24	75	4	14
3	Br	Uveitis	25	7	8	37	9	20
4	Da	Rheumatoid arthritis	30	5	19	51	13	34
			30	11			4	10
5	Co	Rheumatoid arthritis	30	6	10	36	13	13
6	Br	Rheumatoid arthritis	25	13	23	43	10	21
7	Al	Diabetes mellitus	20–50	28	—	—	0	4
8	Pe	Periarteritis nodosa	30	105	—	—	22	3
9	Ka	Acute leukemia	1000	13	33	52	10	18

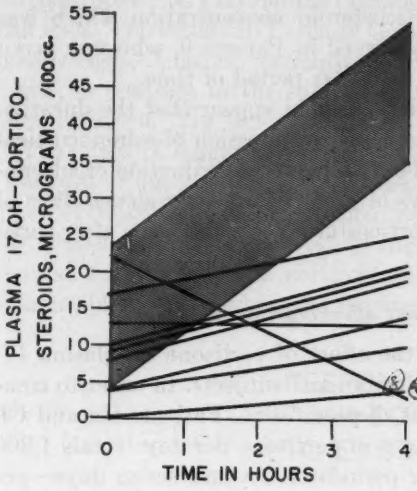

FIG. 2. The effect of prednisone on plasma 17-OH-corticosteroid response to infused corticotropin (ACTH) in patients without adrenocortical disease. The limits of normal response are indicated by the shaded area. Responses of the patients *after* administration of prednisone are shown by the solid lines. Many of the pre-corticotropin levels are normal, but in each case the level of 17-OH-corticosteroids attained after corticotropin is subnormal.

corticotropin in 9 of 12 patients with naturally-occurring pituitary insufficiency.

It is apparent from the data in Table 1 that the degree of adrenocortical suppression bears no constant relation to the dosage of prednisone over the periods of time studied. For example, Patients 1, 2, 3, 5 and 6, who received small doses of prednisone for from six to thirteen days, showed a

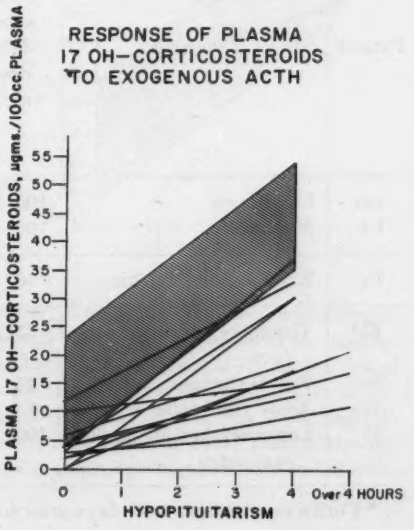

FIG. 3. Response of plasma 17-OH-corticosteroid levels to infused corticotropin (ACTH) in 12 patients with hypopituitarism due to pituitary chromophobe adenoma, craniopharyngioma, and postpartum necrosis. Limits of normal response are indicated by the shaded area. Patients' responses are shown by the solid lines. In 9 of the 12 patients, the increase in corticosteroid is subnormal, presumably because of partial atrophy of the adrenal cortex in association with relatively prolonged deprivation of endogenous corticotropin stimulation.

post-steroid increase in plasma corticosteroid concentration which was quantitatively comparable to that observed in Patient 9, who was given doses up to fifty times as large over a similar period of time.

On the basis of limited observations, it would appear that the duration of steroid administration plays a role in the suppression of adrenocortical response. Patient 4 (Table 1) showed only a moderate reduction of adrenocortical responsiveness after five days of prednisone. After eleven days of this steroid, the increase in plasma corticosteroid concentration after corticotropin was definitely subnormal.

2. *Effect of cortisone in patients without adrenocortical disease*

Table 2 summarizes the data on the effect of cortisone on plasma 17-OH-corticosteroid response to corticotropin in 7 subjects. In order to compare the effect of cortisone with that of prednisone, Patients Go and Ch were given doses of 100–300 milligrams of cortisone per day (totals 1,300 and 700 milligrams, respectively) for periods of ten and seven days—periods comparable to those used for Patients 1–6 in Table 1 who received prednisone. It is apparent in both instances that post-cortisone tests with

TABLE 2. EFFECT OF ORAL CORTISONE THERAPY ON ADRENOCORTICAL RESPONSE TO THE STANDARD CORTICOTROPIN (ACTH) TEST IN ENDOCRINOLOGICALLY NORMAL INDIVIDUALS

Patient	Diagnosis	Cortisone, daily dose (mg.)	Duration of therapy	Plasma 17-OH-corticosteroids (μg./100 cc.)			
				Before cortisone		After cortisone	
				Before ACTH test	After ACTH test	Before ACTH test	After ACTH test
Go	Carcinoma	100–300	10 days	20	37	24	37
Ch	Normal	100	7 days	11	40	23	52
Br	Rheumatoid arthritis	50	2 yrs.	—	—	23	43
Ki*	Thrombocytopenic purpura	37.5	7 mos.	—	—	22	41
Se	Psychoneurosis	25	10 mos.	—	—	8	28
We	Acute rheumatic dever	75	11 mos.	—	—	6	23
Fi	Lupus erythematosus disseminatus	100	12 mos.	—	—	9	15

* Cortisone withdrawn six days prior to study.

corticotropin (ACTH) induced responses comparable to those in the control tests. The results in Patients Go and Ch (Fig. 4) demonstrate that the post-cortisone plasma corticosteroid concentration rose to within normal limits, in contrast to the subnormal responses observed after prednisone administration (Fig. 2).

The effect of longer periods of cortisone administration (from seven to twenty-four months) is seen in Patients Br, Ki, Se, We, and Fi (Table 2). The degree of adrenal suppression induced by cortisone is quite variable (4). Patient Br, who received 50 mg. of the steroid daily for two years showed a normal plasma corticosteroid response, whereas Patient Se (of comparable body weight) who received a smaller dose for a shorter period

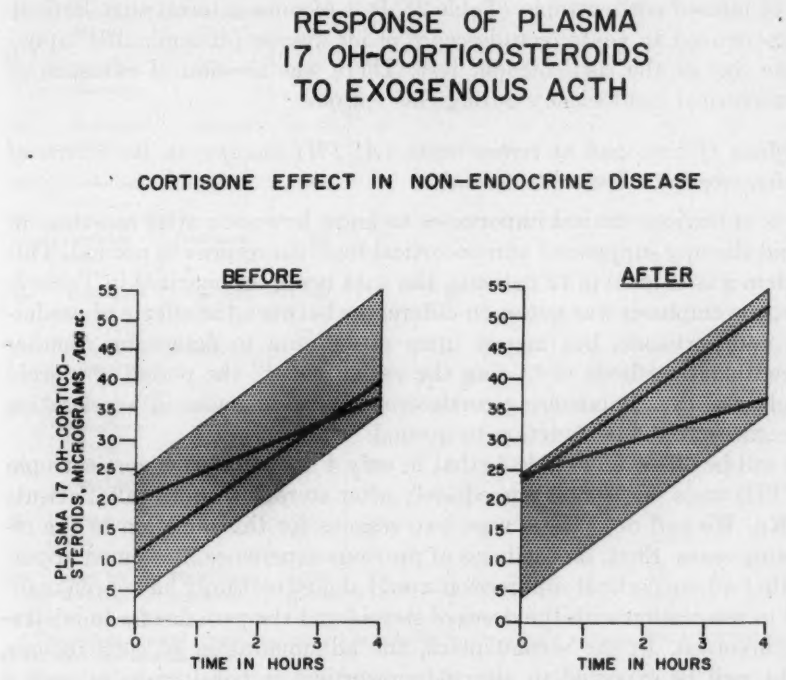

FIG. 4. Effect of cortisone on plasma corticosteroid responses to infused corticotropin (ACTH). Limits of normal response are indicated by the shaded areas of the graphs. Responses of Patients Go and Ch are indicated by solid lines—that of Ch by the line of steeper slope in each graph (11–40 micrograms per 100 cc. before cortisone and 23–52 micrograms per 100 cc. after cortisone); and that of Go by the line of less acute angle in each graph (20–37 micrograms per 100 cc. before cortisone and 24–37 micrograms per 100 cc. after cortisone). Post-cortisone responses in these 2 patients are seen to fall within normal limits.

of time exhibited a subnormal plasma corticosteroid rise after corticotropin. The normal response of Patient Ki is hard to evaluate, since there was a lapse of six days between cortisone withdrawal and corticotropin testing. One does not know whether a corticotropin test performed immediately after cortisone withdrawal would have shown normal or a reduced response. In the latter event, the six-day lapse was apparently sufficient to restore adrenal reactivity to normal. In either case, the patient's normal response to corticotropin on the sixth day correlated well with her normal adrenal status, as illustrated by her uneventful course during and after a cholecystectomy which was performed at this time.

Patients We and Fi, who received larger doses of cortisone (75–100 mg. daily) for approximately one year, showed definite hypo-responsiveness to infused corticotropin (Table 2). It is of some interest that Patient Fi experienced an acute recrudescence of his disease (disseminated lupus) on the day of the corticotropin test. There was no clinical evidence of adrenocortical insufficiency during this episode.

3. *Effects of time and of corticotropin (ACTH) therapy on the return of adrenocortical response to normal*

It is of obvious clinical importance to know how soon after cessation of steroid therapy suppressed adrenocortical function returns to normal. This problem was studied in 12 patients, the data being summarized in Table 3. Here, the emphasis was not upon differences between the effects of prednisone and cortisone, but merely upon attempting to determine whether conventional methods of "tiding the patient over" the period of steroid withdrawal by administering corticotropin were effective in accelerating the return of adrenal function to normal.

It will be observed (Table 3) that in only 4 instances were corticotropin (ACTH) tests performed immediately after steroid withdrawal (Patients Al, Ka, We and Se). There were two reasons for this omission in the remaining cases. First, on the basis of previous experience, it seemed apparent that adrenocortical suppression would almost certainly have been manifest in association with the doses of steroid and the periods of administration involved. In the second place, the administration of corticotropin might well be expected to alter adrenocortical responsiveness in such a way that the effects of time alone on recovery of normal responsiveness would be obscured.

The effect of elapsed time, without corticotropin therapy, was studied in 5 patients (the first 5 in Table 3). In Patient Al, whose plasma corticosteroid response immediately after cessation of prednisone was markedly reduced, a corticotropin test on the fourth day after steroid withdrawal showed an increase in adrenocortical responsiveness which, however, was

TABLE 3. INFLUENCE OF TIME AND OF TREATMENT WITH CORTICOTROPIN IN RESTORING TO NORMAL THE ADRENOCORTICAL HYPO-RESPONSIVENESS WHICH OCCURS AFTER STEROID WITHDRAWAL. CORTICOTROPIN (ACTH) TESTS WERE PERFORMED IN THE STANDARD MANNER (*see* text)

Patient	Diagnosis	Steroid	Daily dose (mg.)	Duration of therapy (days)	Plasma 17-OH-CS (μg./100 cc.) response immediately after steroid therapy		Time elapsed after withdrawal (days)	ACTH (mg. per day)	Plasma 17-OH-CS (μg./100 cc.) response after ACTH therapy	
					Before ACTH test	After ACTH test			Before ACTH test	After ACTH test
Al	Diabetes mellitus	Prednisone	20–50	28	0	4	4	0	4	26
Ri	Rheumatoid arthritis	Prednisone	30	25	—	—	4	0	13	31
Gr	Lupus erythematosus	Prednisone	50	27	—	—	3	0	16	44
Ca	Rheumatoid arthritis	Prednisone	15–30	90	—	—	7	0	25	41
Ba	Psychoneurosis	Cortisone	50	240	—	—	2	0	20	33
Alx	Acute leukemia	Prednisone	1,000	17	—	—	1	25 5 days	15	49
Ka	Acute leukemia	Prednisone	1,000	13	10	18	3	40 3 days	9	47
Sh	Psoriatic arthritis	Prednisone	40	76	—	—	4	40 3 days	21	59
O'D	Lupus erythematosus	Prednisone	10–100	180	—	—	6	25 6 days	14	46
We	Acute rheumatic fever	Cortisone	75	330	6	23	10	20 10 days	5	20
Se	Psychoneurosis	Cortisone	25	300	8	28	13	20 3 days	21	35
Cal	Acute leukemia	Prednisolone Cortisone*	1,000 50–200	9 6	— —	— —	8	40 3 days	21	62

* This patient received prednisolone followed immediately by the course of cortisone. The ACTH test was performed eight days after the latter.

still subnormal. Similarly, in Patient Ri, who received prednisone for a comparable period, a corticotropin test on the fourth day after withdrawal of prednisone revealed a somewhat reduced rise in plasma corticosteroid levels. In contrast, Patient Gr, similarly treated, showed normal adrenocortical reactivity on the third post-steroid day. Patient Ca, treated with prednisone for ninety days, had normal adrenal reactivity one week after steroid withdrawal. Patient Ba, who received cortisone for eight months,

showed a very slight degree of adrenal suppression two days after cessation of the hormone. These findings confirm those of Sprague and his colleagues (4) concerning the wide individual variations in duration of adrenocortical insufficiency following long-term treatment with steroids.

In the remaining 7 patients (Table 3), tested one to thirteen days after withdrawal of prednisone or cortisone, corticotropin (ACTH) was administered in an effort to accelerate the return of normal adrenocortical function. Doses employed were 20–40 mg. of ACTH Gel daily by the intramuscular route for three to ten days, except in Patient O'D who received ACTH intravenously. The data in Table 3 (last two columns) indicate that in all but 1 instance (Patient We) adrenocortical response in terms of plasma corticosteroid rise was normal after the course of corticotropin therapy. In those patients (Alx, Ka, Sh and O'D) in whom the elapsed time after steroid withdrawal was comparable to that in the patients not given corticotropin, post-corticotropin adrenal response was normal in all 4 instances in contrast to that in the untreated patients, only 2 of whom showed a normal plasma corticosteroid rise. Although this constitutes suggestive evidence that corticotropin therapy did accelerate the return of adrenocortical responsiveness to normal, the small number of cases and the individual variations observed (Al, Ri and Gr) do not permit one to state that the acceleration is conclusively proved.

Perhaps the most interesting result was that observed in Patient We. In this 11-year-old child, ten days of treatment with corticotropin failed to bring about normal reactivity of the adrenal cortex. The rise in plasma corticosteroid concentration after corticotropin treatment remained subnormal, and was quantitatively similar to that immediately following steroid withdrawal. It may be significant that this patient had received the longest course of steroid therapy in the group.

In order to determine the stability of the normal adrenocortical reactivity which appeared to follow a course of corticotropin therapy, 1 patient (O'D) was tested again seven days later—thirteen days after steroid withdrawal. After six days of intravenous corticotropin therapy, a corticotropin test showed a rise in the plasma corticosteroid level from 14 micrograms to 46 micrograms per 100 cc., which is a normal response (Table 3). Seven days later, without further corticotropin treatment, the test revealed a rise in the plasma corticosteroid level from 10 micrograms to 31 micrograms per 100 cc., which is slightly below the lower limit of the normal response (Fig. 1).

In all these patients, other indices of adrenocortical adequacy, such as well-being, appetite, weight, blood pressure, and serum glucose, sodium and potassium levels were followed. In no case was there clear-cut objective evidence of adrenocortical insufficiency during the period of demonstrated

adrenal hypo-responsiveness. In Patient Gr there was a five-day period of tachycardia coincident with cessation of prednisone therapy. Patients Ka and Se[2] exhibited subjective feelings of extreme weakness, reversed in the case of Se by steroid and parenteral fluid therapy. In none of these patients were there measurable objective changes characteristic of adrenal crisis. These findings again confirm those of Sprague and his co-workers (4).

4. *Effect of prednisone and cortisone in late pregnancy*

It has been shown previously (12) that the adrenal cortex in late pregnancy is "hyper-responsive." Concentrations of plasma 17-OH-corticosteriods attained after corticotropin testing in the third trimester of pregnancy are comparable in magnitude to those attained by patients with Cushing's syndrome due to bilateral adrenal hyperplasia (12). Since situations may arise which make it necessary to administer adrenocortical steroids to pregnant women (*e.g.*, disseminated mesenchymal diseases), it seemed important to learn whether the hyper-responsive adrenal cortex of late pregnancy could be suppressed, as in the normal subject, or whether it was relatively resistant to inhibition by exogenous steroid, as observed in this laboratory in patients with Cushing's syndrome due to bilateral adrenal hyperplasia (15). Accordingly, 2 women who were in the eighth month of uncomplicated pregnancy were given prednisone, 25 mg. per day, for seven days, the dose and time being similar to those employed in the normal subjects (Patients 1–6, Table 1). In both cases (Patients Gu and Ha), as seen in Table 4, there was significant reduction in the plasma 17-OH-corticosteroid response to corticotropin after prednisone therapy. Patient Gu's adrenocortical response remained slightly excessive, whereas Patient Ha's post-prednisone response fell below the range observed in the 12 patients in late pregnancy studied to date. The magnitude of the response was in the normal range, in contrast to the subnormal post-prednisone responses occurring in the normal subjects (Fig. 2).

A third patient (Pa, Table 4), given prednisone for severe asthma in doses averaging 40 mg. daily over a longer period (thirty days), was tested. The plasma corticosteroid level showed no significant change after intravenous administration of corticotropin.

Cortisone, administered for long periods, is also apparently capable of reducing the hyper-reactivity to corticotropin which occurs late in preg-

[2] It is worth mentioning that in Patient Se, who had been suspected of having Addison's disease and had been treated elsewhere for this condition, there was a response of the plasma corticosteroid level to corticotropin testing. Addisonians usually show no rise in plasma corticosteroids after administration of corticotropin (12). Patient Se is now doing well without steroid therapy, her lack of energy having responded satisfactorily to amphetamine.

TABLE 4. EFFECT OF PREDNISONE AND CORTISONE ON THE ADRENOCORTICAL "HYPER-RESPONSIVENESS" OF LATE PREGNANCY. THE CORTICOTROPIN (ACTH) TEST WAS PERFORMED IN THE STANDARD MANNER

Patient	Steroid	Daily dose (mg.)	Duration of therapy (days)	Plasma 17-OH-corticosteroids (μg./100 cc.)			
				Before steroid		After steroid	
				Before ACTH test	After ACTH test	Before ACTH test	After ACTH test
Gu	Prednisone	25	7	30	90*	36	64
Ha	Prednisone	25	7	33	84*	22	51
Pa	Prednisone	40	30	—	—	50	51
Ma	Cortisone	100	100	—	—	24	68
Ya	Cortisone	75	60	—	—	59†	52

* In 9 untreated women in the third trimester of pregnancy, control plasma 17-OH-corticosteroid values ranged from 30 to 52 micrograms per 100 cc., rising to 79–111 micrograms per 100 cc. after the standard corticotropin test.

† Specimen obtained only three hours after cortisone (25 mg.), so that this high value is in part due to inclusion of absorbed exogenous cortisone in the measurement.

nancy. As shown in Table 4 (Patients Ma and Ya), cortisone therapy for periods of months resulted in adrenocortical responses somewhat less than those found in this laboratory in a group of 12 women similarly tested in the third trimester. Since control corticotropin tests before cortisone therapy were not obtained in these 2 women, no statement can be made about the degree of suppression achieved, and since the periods of administration of prednisone and of cortisone were dissimilar, no accurate comparison can be made of the relative efficacy of the two steroids in causing adrenocortical suppression in the pregnant state.

DISCUSSION

The results of this study appear to support the thesis of Sayers (3), which states that a parallelism exists between the "anti-inflammatory" potency of a steroid and its ability to inhibit release of corticotropin by the anterior pituitary. Using the response of plasma 17-OH-corticosteroid levels to a standardized intravenous corticotropin (ACTH) test as the measure of adrenocortical reactivity (12), evidence has been obtained suggesting that prednisone is four or more times as effective as cortisone in suppressing endogenous pituitary-adrenal function. Prednisone in 25-mg. doses induced adrenocortical hypo-responsiveness after seven days of administration, whereas doses of cortisone four times as great failed to bring

about such a suppression over comparable periods. Previous studies on the relative anti-inflammatory effects of the two steroids have also shown prednisone to be about four times as potent as cortisone in controlling the symptoms of such disorders as rheumatoid arthritis (8).

A word should be said about the significance of the post-steroid changes in adrenocortical response. One might ask—Are the reductions in responsiveness real? How great is the variation in plasma corticosteroid response to a standard corticotropin test in a single individual tested on separate occasions? It has been shown by Eik-Nes and his co-workers (16) that this variation may be considerable. However, the reported day-to-day changes appear to vary within a normal range (16). The post-steroid adrenocortical responses, described here as "suppressed" responses (for example, those occurring after prednisone administration) are clearly below the range of normal (Table 1, Fig. 2).

The question of how soon after steroid withdrawal adrenocortical reactivity returns to normal has obvious practical significance in patient management. It has been reported that a "hypo-adrenal" state may persist for several months after cessation of hormone therapy (4, 6a, 6b, 7), and that normal adrenal reactivity may be restored by repeated doses of intravenous corticotropin within sixty hours in some cases (17). From the data presented here, it is clear that a period of four days following even relatively short-term prednisone treatment may not always be sufficient to allow complete recovery of adrenal responsiveness to corticotropin. It is also clear that although no demonstrable adrenal suppression appears after short-term cortisone administration, adrenal reactivity following longer periods of cortisone is highly variable from patient to patient. There is suggestive but not conclusive evidence that corticotropin therapy, after withdrawal of either steroid, accelerates the return of adrenocortical response toward normal. It is suggested that the degree of refractoriness to corticotropin may be more closely related to the duration of steroid therapy (Patient We, Table 3) than to the size of the dose of steroid (Patient Ka, Table 3).

It should be appreciated that none of the patients studied showed objective evidence of adrenal insufficiency during or after the periods of steroid withdrawal. In certain instances, one wonders if the test dose of corticotropin played some part in preventing the development of clinical adrenal crisis. This seems unlikely in view of the demonstrated rather small stimulating effect of corticotropin (*e.g.*, after prednisone, Table 1). Moreover, as Bayliss (18) has pointed out, plasma corticosteroid levels, even in untreated normal subjects, remain elevated for only short periods after intravenous corticotropin.

A more important question is—Would these patients with suppressed

adrenocortical reactivity have shown signs of adrenal insufficiency if a severe and prolonged stressful stimulus such as surgery had been imposed upon them during the period after steroid withdrawal? This question cannot be answered definitely on the basis of the data reported here. The index used (response of the adrenal cortex to corticotropin) tests the sensitivity of the adrenal only. No data are available which permit a test of the ability of the pituitary to respond. Lack of pituitary response may well be the crucial factor in determining the functional integrity of the pituitary-adrenal system in its reaction to a stimulus in such circumstances as these. Until such data are forthcoming, it would seem well-advised to assume that the findings reported here and elsewhere (4, 6a, 7) imply some degree of insufficiency of the pituitary-adrenal system; thus, in the event of impending trauma (*e.g.*, a surgical procedure) exogenous steroid should be supplied. In that event, it may be expected that administration of corticotropin alone would yield only small and transient titers of endogenous adrenocortical hormone.

With respect to the effects of steroid treatment on the adrenal cortices of pregnant women, the situation is not clear. It is of considerable interest that the reportedly enlarged (literature reviewed by Smith (19)) and "hyper-responsive" adrenal cortex (12) of late pregnancy can be suppressed to some extent by steroid hormones. This susceptibility to suppression by steroid is in contrast to the refractoriness to suppression of the adrenal cortex in 6 patients with Cushing's syndrome due to bilateral adrenal hyperplasia. In these hyper-adrenal patients, large doses of prednisone failed to reduce the over-reactivity of the gland in response to corticotropin (15). No explanation for this difference between the adrenal cortex of late pregnancy and that of Cushing's syndrome can be advanced at the present time. It is of further interest that the plasma corticosteroid responses to corticotropin in the pregnant women treated with prednisone did not reach the subnormal levels observed in normal individuals (Tables 1 and 4). The significance of these observations will not be clear until there is more definite identification of the substances which make up the increased Porter-Silber reactive material found in late pregnancy. This material has been assumed to be in large part hydrocortisone (20).

SUMMARY

1. A study of the comparative effects of orally administered prednisone and cortisone on adrenocortical responsiveness has been carried out in 29 patients. Plasma 17-hydroxycorticosteroid response to a standardized intravenous corticotropin (ACTH) test was used as the index of adrenal reactivity.

2. Prednisone, administered for periods of one to two weeks, appears to

be four or more times as effective as similarly administered cortisone in suppressing adrenocortical responsiveness.

3. There is evidence suggesting that treatment with corticotropin may accelerate the return of post-steroid adrenocortical reactivity from the subnormal to the normal state. However, restoration of normal adrenal responsiveness to administered corticotropin should not be considered as implying a normal capacity of the pituitary to react to a sustained stressful stimulus.

4. The hyper-reactive adrenal cortex of late pregnancy can be partially suppressed by administration of prednisone, unlike the adrenal cortex of Cushing's syndrome due to bilateral adrenal hyperplasia, which is refractory to this suppressive effect.

Acknowledgment

The authors are grateful for the valuable technical assistance of Miss Elsie Ewen.

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INCREASED URINARY ESTROGEN AND 17-KETOSTEROID EXCRETION ASSOCIATED WITH ADRENAL ADENOMA. ISOLATION OF CHOLESTANE-DIONE-3,6 FROM THE TUMOR

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MANY cases of adrenal tumor accompanied by increased urinary excretion of estrogens and 17-ketosteroids have been reported. We should like to present another interesting case, in which we had the opportunity to make thorough clinical and hormonal investigations, and to isolate cholestane-dione-3,6 from the tumor.

CASE REPORT

Anamnesis

The patient, a woman of 30, entered the clinic in November 1954. After the menarche at 13 she had menstruated regularly every four weeks. She was married in 1944 and underwent a partus immaturus in the same year; the etiology was unknown. Ten years previously oligomenorrhea and hirsutism had been observed and both had steadily increased. For two years she had had amenorrhea. Sexual libido was normal.

Clinical findings and tests

Examination of the patient revealed hypertrichosis of the extremities and breasts, a marked growth of hair on the face, and frontal baldness of the scalp (Figs. 1 and 2). The voice was rough and low pitched. There was no hypertonia. The uterus was small. No endometrial samples were obtained by curettage. The clitoris was hypertrophied. No tumor could be felt by examination under narcosis. The roentgenologic findings (intravenous pyelogram) showed the right adrenal to be in a normal position, whereas the left adrenal was lower than normal. The results of both the Thorn test and the Robinson-Power-Kepler test were normal. An exploratory laparotomy showed ovaries of normal size; they were not atrophic. Small follicle cysts were discovered upon histologic examination. No testes were found.

General laboratory data

No abnormality was discovered by chemical and microscopic examination of the urine or by examination of the blood picture. The results of blood chemical analyses were normal, apart from a slight increase in the gamma-globulin level and a transient elevation of the serum bilirubin level. The values for concentration of nonprotein nitrogen, urea, total protein, pyruvic acid, sodium, chloride, potassium, calcium and blood sugar and for the thymol-turbidity test were within the normal range. Analysis by paper chromatography of the amino acids yielded normal results.

Received December 9, 1955.

FIGURE 1 FIGURE 2

Hormone determinations

Figure 3 summarizes the results of urinary hormone assays. The excretion of 17-ketosteroids was unusually high—300 mg. and 225 mg. per twenty-four hours (normal range, 7 to 16 mg.). Androgen excretion, determined by bioassay (capon comb-growth test[1]), was also excessive, being equivalent to 40–50 mg. per twenty-four hours of androsterone (normal range, 3 to 10 mg.). The phenol steroid excretion showed a marked increase. It was estimated (fluorescence method) to be 600 I.U. per twenty-four hours (normal range, 50 to 450 I.U.). Estrogen excretion (bioassay) was elevated—280 R.U. per twenty-four hours (normal range, 25 to 125 R.U.). The urinary 17-hydroxycorticosteroids were increased—28.8 mg. and 28.0 mg. per twenty-four hours (normal range, 4 to 7 mg.). The amount of follicle-stimulating hormone (FSH) excreted was 10 uterine R.U. per twenty-four hours, a value lower than normal (20 to 40 uterine R.U.).

Papanicolaou tests were carried out on the vaginal smears, but no estrogen effect could be observed; only basal cells were found (Fig. 4).

The result of the chromatographic differentiation of the excreted neutral 17-ketosteroids is represented in Figure 6. The constituents of the different fractions are:

Fraction I: artifacts
Fraction II: *i*-androstane-ol-6-one-17
Fraction III: dehydroisoandrosterone, isoandrosterone

[1] This test was kindly performed by Ciba A. G., Basle.

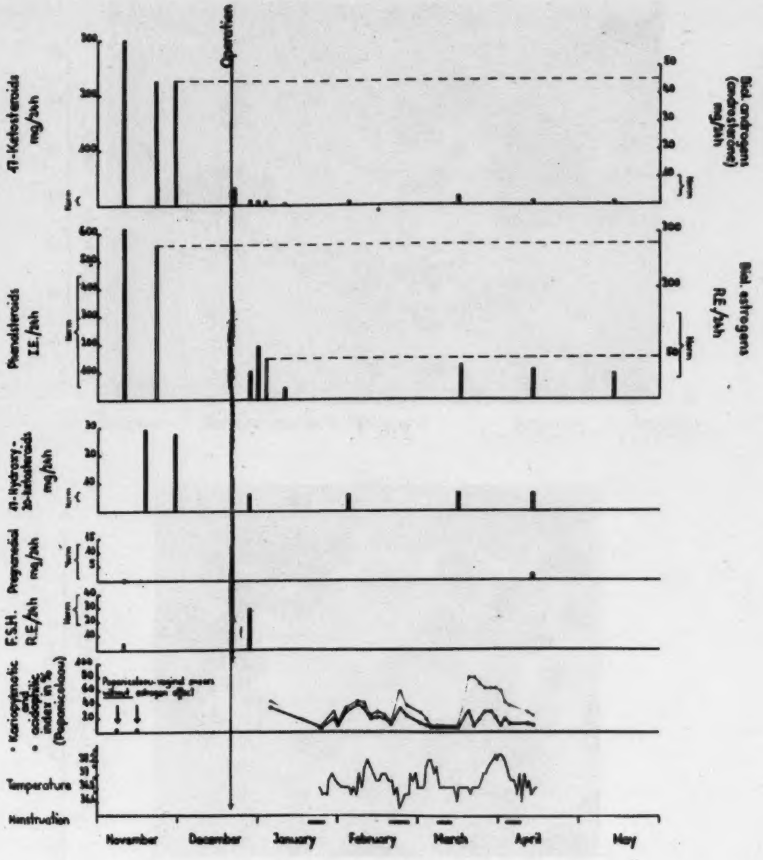

FIG. 3. Hormone excretion and vaginal smears.

Fraction IV: androsterone, Δ^5-androstene-ol-(3α)-one-17
Fraction V: etiocholane-ol-(3α)-one-17, Δ^9-etiocholene-ol-(3α)-one-17
Fraction VI: 11-hydroxyandrosterone
Fraction VII: 11-hydroxyetiocholane-ol-(3α)-one-17
Fraction VIII: unknown 17-ketosteroids.

Diagnosis

Chromatography clearly showed that the compounds of fraction III, consisting mainly of the β-steroids dehydroisoandrosterone and isoandrosterone, were, in particular, excreted in increased quantities. The source of these compounds is most probably the adrenal cortex. The ratio of fraction III to fraction IV is normally about 0.5 for healthy women. In our patient the ratio was about 6.0. Experience has shown that an absolute or relative increase of fraction III is mostly to be found in cases of adrenal tumor or hyperplasia of the adrenal cortex.

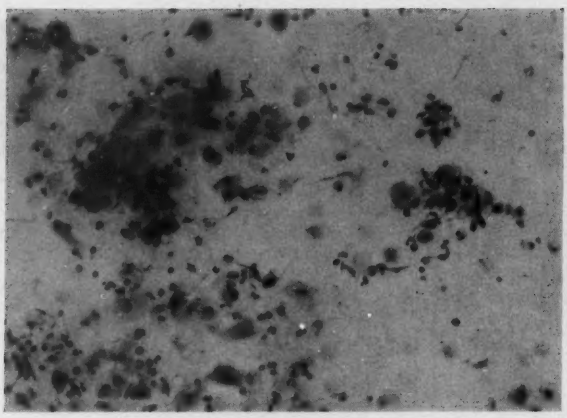

FIG. 4.

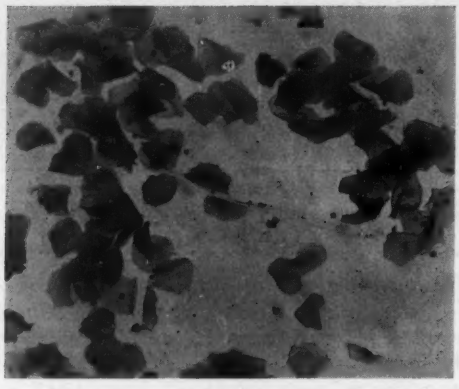

FIG. 5

Vaginal smears (Papanicolaou):
FIG. 4. Before removal of adrenal tumor.
FIG. 5. After removal of adrenal tumor.

A summary of the results of the hormone determinations indicated a high-grade hyperfunction of the adrenal cortex, which was causing a considerable disturbance of the steroid metabolism. The unusually high androgen and estrogen production was hindering hormone excretion from the pituitary gland and therefore ovarian function had ceased. The patient was in a state of "chemical castration." In spite of the increased estrogen excretion, no sign of an estrogen effect was found in the vaginal smears, probably because of the large amount of androgen present. In order to determine whether the adrenal abnormality was a tumor or a hyperplasia of the cortex, the effect of cortisone on 17-ketosteroid excretion should have been studied. Unfortunately, this experiment was not feasible. The results of the hormone determinations together with the

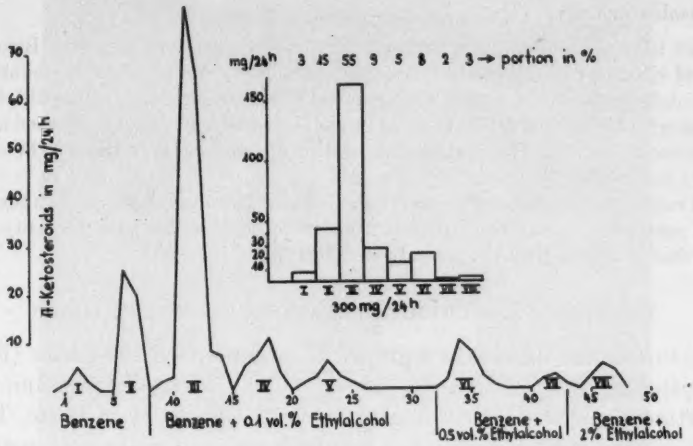
17-Ketosteroids in mg/24 h
mg/24h 3 15 55 9 5 8 2 3 → portion in %
300 mg/24 h
Benzene
Benzene + 0.1 vol.% Ethylalcohol
Benzene + 0.5 vol.% Ethylalcohol
Benzene + 2% Ethylalcohol

FIG. 6

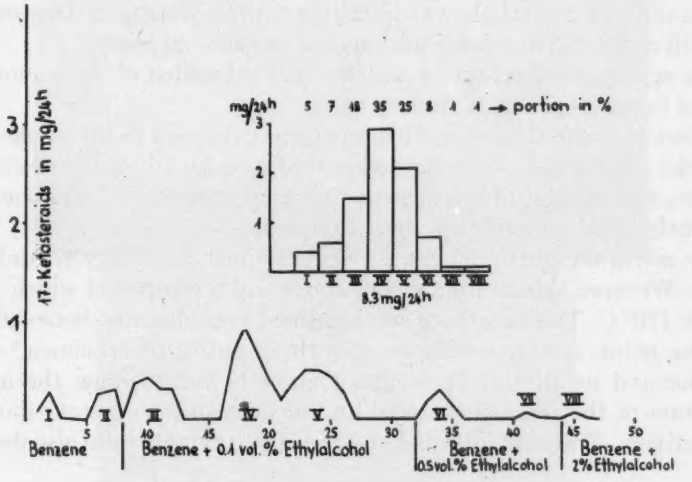
17-Ketosteroids in mg/24 h
mg/24h 5 7 18 35 25 8 4 4 → portion in %
8.3 mg/24 h
Benzene
Benzene + 0.1 vol.% Ethylalcohol
Benzene + 0.5 vol.% Ethylalcohol
Benzene + 2% Ethylalcohol

FIG. 7

Chromatographic differentiation of the 17-ketosteroids:
FIG. 6. Before removal of adrenal tumor.
FIG. 7. After removal of adrenal tumor.

anamnesis and the development of the various disorders indicated the presence of an adrenal tumor.

The operation was performed by Prof. Heusser and Prof. Wenner and a well defined tumor of the left adrenal was removed.

Postoperative findings

Apart from a pneumothorax, no postoperative complications occurred. Blood pressure and electrolyte concentration remained completely normal. The hormone values immediately decreased to normal after removal of the tumor (Fig. 3). Repetition of the chromatographic differentiation of the neutral 17-ketosteroids showed a marked decrease in fractions II and III. The quantitative relation of fraction III to fraction IV changed from 6.1 to 0.5 (Fig. 7).

The vaginal smears became normal (Fig. 5). Facial hair diminished, and on the thirty-second postoperative day the first menstruation occurred without the necessity of hormonal therapy. Since then the patient has menstruated regularly.

HISTOLOGIC AND CHEMICAL EXAMINATION OF THE TUMOR

The tumor was the size of a grapefruit and weighed 180 grams (Fig. 8). The histologic examination was carried out by Prof. Werthemann in the Department of Pathological Anatomy of the University of Basle. The report described the tumor as being brownish yellow in color, soft, marrowy, nearly homogenous, and slightly flabby. Some parts were blood stained. There were marked intercalations of adipose tissue and small localized calcifications. Figure 10 shows a histologic section. Diagnosis: Degenerated, but still rather differentiated adenoma of the adrenal cortex.

The experimental set-up for the chemical extraction of the tumor is described in the Appendix of this report.

A part of the fresh tumor (79 grams) was extracted in the research laboratories of Ciba A. G. by a method proved good for adrenal cortex investigations, and examined for cortisone, hydrocortisone and aldosterone.[2] Only extremely small quantities of these compounds were discovered.

The rest of the tumor (95 grams) was examined chemically in our laboratories. We were able to isolate cholesterol and a compound which melted at 168–170° C. This substance was identified as cholestane-dione-3,6 by its melting point, mixed melting point with an authentic specimen,[3] optical rotation and its infrared spectrum. Figures 11 and 12 show the infrared spectrum of the isolated material and of authentic cholestane-dione-3,6, respectively. Traces of estradiol and of 17-ketosteroids were also detected.

DISCUSSION

The amounts of steroid compounds (calculated for the whole 180-gram tumor) which were isolated or detected were small. The various substances

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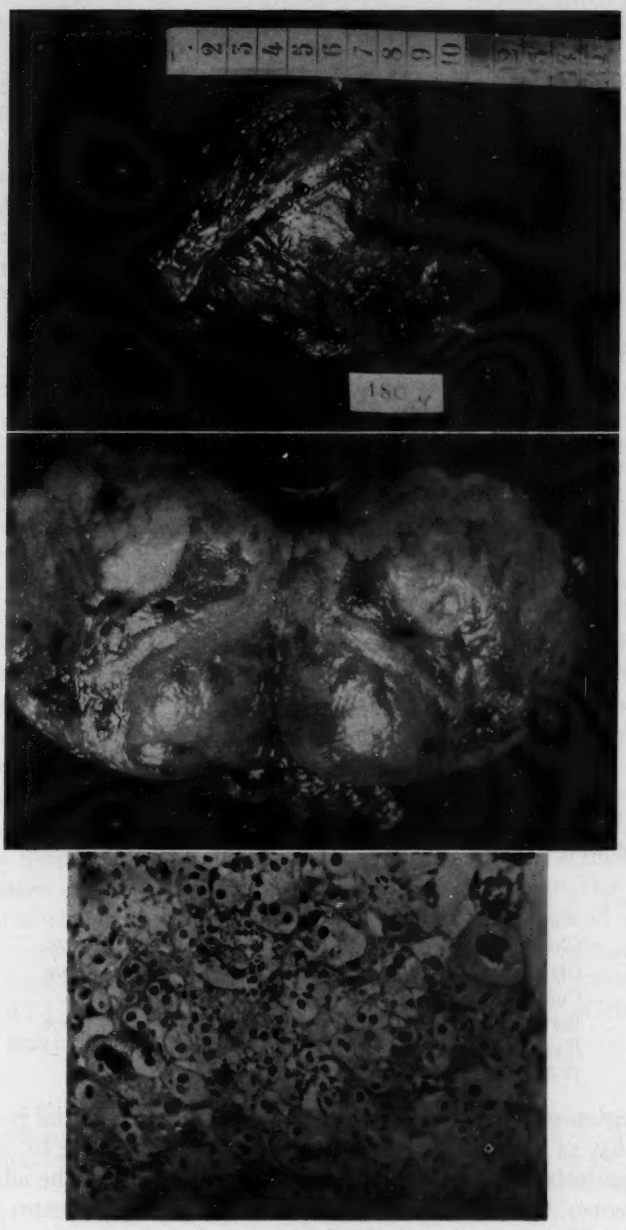
[2] Dr. Wettstein and Dr. Neher kindly made the estimations of cortisone, hydrocortisone and aldosterone in the tumor material.

[3] Our thanks are due to Prof. Reichstein and Prof. Prelog for a supply of cholestane-dione-3,6 and to Dr. Neher and Dr. Zoller, who kindly carried out the infrared spectrum analysis.



Adrenocortical adenoma

FIG. 8 (top). Intact.

FIG. 9 (center). Cut surface.

FIG. 10 (below). Histologic section.

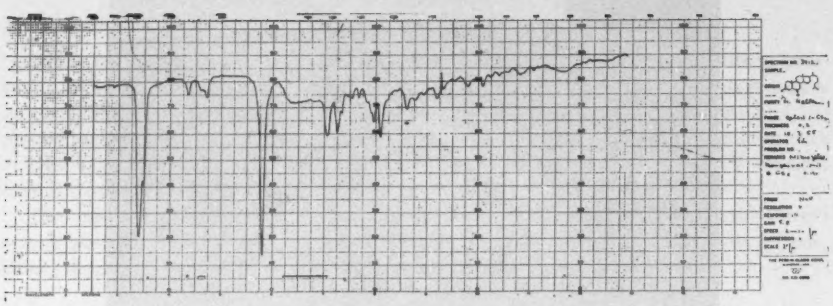


FIG. 11

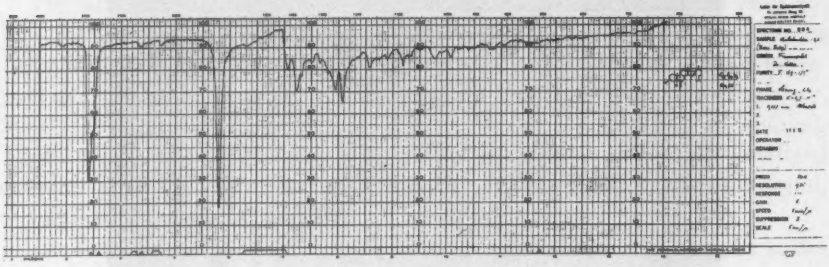


FIG. 12

Infra-red spectra:

FIG. 11. Of material isolated from the adrenocortical tumor.
FIG. 12. Of authentic cholestane-dione-3,6.

recovered were:

Compound	*Approx. amount*
Aldosterone	1γ
Cholestane-dione-3,6	6 mg.
Cholesterol	40 mg.
Cortisone	6γ
Estradiol	Trace
Hydrocortisone	1γ
17-Ketosteroids	6γ

The existence of cholestane-dione-3,6 in the tumor material is especially noteworthy, as this compound has never before (according to our knowledge) been isolated from normal or degenerated tissue of the adrenal cortex. Moreover, the amount of this steroid was strikingly greater than that of the other identified steroids. Cholestane-dione-3,6 was isolated and identified for the first time from organic material (pig testis) by Prelog, Tagmann, Lieberman and Ruzicka (1). Its formation from cholesterol by autoxydation during the extraction and working-up process is very un-

likely. Its presence in the testis and in a tumor of our type, which surely produced large amounts of androgens, makes it possible that cholestane-dione-3,6 may play a certain role in the synthesis of androgens from cholesterol. Dingemanse and her co-workers (2) as well as Barton and Klyne (3) were able to isolate and identify *i*-androstane-ol-6-one-17 from urinary extracts. This steroid metabolite, hydroxylated in position 6, seems to be excreted in larger amounts by men than by women. It occurs in fraction II when the neutral 17-ketosteroids are chromatographically differentiated by the method described by Dingemanse. This fraction was definitely increased in the urine of the patient but diminished after removal of the tumor. Possibly a relationship exists between the urinary steroids hydroxylated in position 6 and the 6-ketosteroids in the testis and the adrenal cortex.

SUMMARY

A 30-year-old woman with an adrenocortical adenoma had hypertrichosis of the extremities, breasts and face, frontal scalp baldness, a hoarse low-pitched voice, clitoral hypertrophy, a small uterus and amenorrhea. There was no hypertonia. Roentgenologic examination showed the left adrenal to be lower than normal. The results of the Thorn test, the Robinson-Power-Kepler test and general laboratory examinations were normal.

Steroid hormone metabolism was markedly disturbed. There was increased urinary excretion of 17-ketosteroids (predominantly dehydroisoandrosterone) and of estrogens. In spite of the raised estrogen level, no sign of an estrogen effect was found in the vaginal smears. After removal of the adrenocortical tumor, the urinary hormone values immediately decreased to normal levels. Facial hair diminished, and on the thirty-second postoperative day the first menstruation occurred without the necessity of hormonal therapy. Since then the patient has menstruated regularly.

The tumor weighed 180 grams and was examined chemically and histologically. Besides small amounts of aldosterone, cortisone, hydrocortisone, estradiol, 17-ketosteroids and cholesterol, a small quantity of cholestane-dione-3,6 was isolated and identified.

Acknowledgment

The helpful advice of Prof. A. Werthemann, Prof. R. Wenner and Dr. A. Hauser is gratefully acknowledged. We also wish to express our appreciation to Dr. H. Zoller, Dr. L. Kunz, Mrs. R. Berde, Miss H. Hertig and Mr. F. Waldmeier for their excellent technical assistance.

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APPENDIX

Hormone determination

The determination of urinary hormone excretion was made by the routine clinical methods used in our laboratory. The technic for total neutral 17-ketosteroids was a modification of that described by Drekter and his co-workers (4). The total "phenol steroids" were determined by a chemical method described by Keller (5). For the bioassay of estrogens the "phenolic fraction" was obtained by extracting with 1N KOH from the CCl_4-extract. This technic separates the true estrogens from the androgens and is necessary, since dehydroisoandrosterone and certain other androgens have been shown to have a vaginal cornifying effect. Bioassay for estrogens was performed at selected levels in castrated rats, using a modification of the Allen-Doisy vaginal smear technic. 20-Ketosteroids were determined by a modified method described by Gornall and Macdonald (6). Bioassay for FSH was carried out in rats, using the slightly modified technic of Levin and Tyndale (7). Pregnanediol estimation was performed by a modified method proposed by Huber (8). The technic of Dingemanse and co-workers (2) was applied for the chromatographic differentiation of the excreted neutral 17-ketosteroids. Before operation one-fiftieth of a 24-hour urine specimen was extracted and the extract was chromatographed on 15 grams of alumina. After removal of the tumor, three-fourths of a 24-urine specimen was extracted and examined by chromatography.

Examination of the tumor (180 grams)

A part of the tumor (79 grams) was extracted in the research laboratories of Ciba A.G. and examined for cortisone, hydrocortisone and aldosterone. The fresh tumor material was homogenized for twelve minutes in 80 ml. of NaCl-solution (0.9 per cent) with the addition of a little ice; the mixture was then poured into 2,000 ml. of acetone and

kept over night. After filtration and three washings of the residue on the filter with 500 ml. of acetone each time, the filtrate was concentrated under reduced pressure and the remaining aqueous suspension degreased with petroleum ether. Extraction with ethylene chloride followed. A part of the ethylene chloride extract (50.2 mg., total) was analyzed by paper chromatography according to a method described by Wettstein, Kahnt and Neher for corticosteroid estimation (except aldosterone). Aldosterone was estimated by the technic described by Wettstein *et al.* (9). Only very small amounts of cortisone (2.5γ), hydrocortisone (0.5γ) and aldosterone (0.5γ) were detected.

The rest of the tumor was examined chemically in our laboratory. The material was divided into small pieces and finely pulverized with sea sand. The extraction process, mainly in order to isolate estrogens, is described schematically in the Tables A, B and C.

Negative results were obtained after testing the aqueous phases B (Table B) and D (Table A) for the possible presence of 17-ketosteroids (Zimmermann reaction) and estrogens (fluorescence test and paper chromatography).

In the tumor residue C (Table C), traces of estradiol were detected by means of paper chromatography (Bush (10)). The Zimmermann reaction revealed approximately 3γ of 17-ketosteroids.

The working up of the ether phase A (Table A), after separation from the aqueous phase D, resulted in 105 mg. of a slightly yellowish, crystalline residue (ether phase E, Table A), which was chromatographed on alumina (3 Gm.). Elution with petroleum ether yielded uncrystallizable, yellowish oils (30 mg.). Elution with benzene yielded a solid residue (70 mg.) consisting of an admixture of cholesterol with another compound, which was difficult to separate by chromatography. This mixture was dissolved in pyridine (2 ml.) and acetic anhydride (1 ml.) and kept for eighteen hours at room temperature. The solvent was removed at reduced pressure and the ethereal extract of the crude acetylation product was washed until neutral, dried over Na_2SO_4, and the ether evaporated. The residue was chromatographed on alumina (3 Gm.). Elution with petroleum ether-benzene, followed by crystallization from methanol, resulted in pure cholesterol-acetate (22 mg.) with a melting point of 113–115° C., which was not depressed on admixture with an authentic specimen. Elution with benzene, followed by crystallization from ether-petroleum ether and chloroform-petroleum ether yielded needles (3.1 mg.), which melted at 168–170° C. This compound was identified as cholestane-dione-3,6 by its melting point; mixed melting point with an authentic specimen; optical rotation $[\alpha]^{20}_D = +12° \pm 4°$ (c = 0.307 in chloroform); and its infra-red spectrum (Fig. 11) which was determined on the material recovered from the optical rotation test.

The melting points were taken on a Kofler block. The alumina used for the chromatography was from Riedel-de Haën.

TABLE A

The rest of the tumor (70 Gm.) was divided into small pieces and finely pulverized with sea sand.

Extracted with 2×500 ml. 80% EtOH.
Extracted with 2×500 ml. 95% EtOH.
Extracted with 2×500 ml. BuOH.

[1]

Combined extracts evaporated to dryness at a reduced pressure and residue suspended in 150 ml. H_2O. Extracted with 4×500 ml. ether.

Residue C
Starting material for TABLE C

TABLE A—*continued*

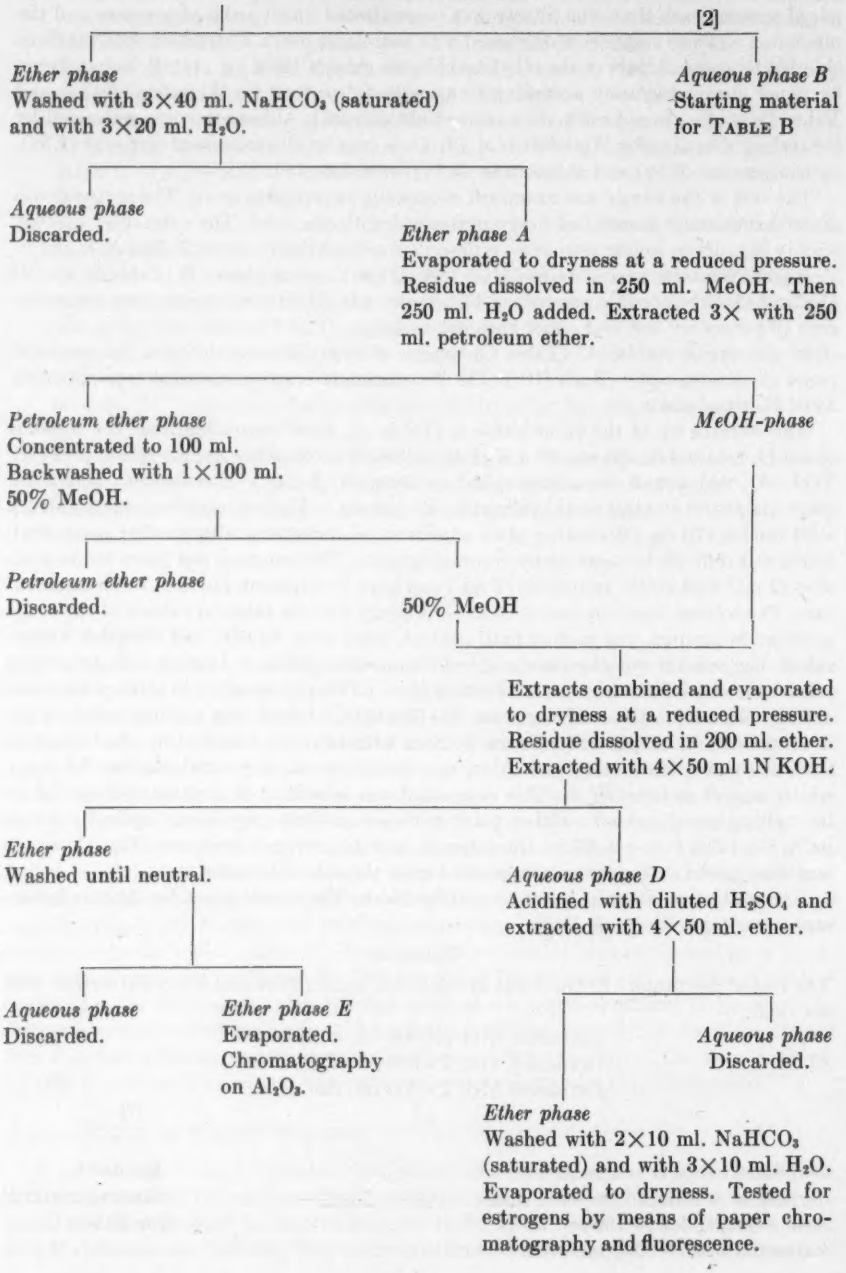
[2]
Ether phase
Washed with 3×40 ml. $NaHCO_3$ (saturated) and with 3×20 ml. H_2O.
Aqueous phase B
Starting material for TABLE B
Aqueous phase
Discarded.
Ether phase A
Evaporated to dryness at a reduced pressure. Residue dissolved in 250 ml. MeOH. Then 250 ml. H_2O added. Extracted 3× with 250 ml. petroleum ether.
Petroleum ether phase
Concentrated to 100 ml. Backwashed with 1×100 ml. 50% MeOH.
MeOH-phase
Petroleum ether phase
Discarded.
50% MeOH
Extracts combined and evaporated to dryness at a reduced pressure. Residue dissolved in 200 ml. ether. Extracted with 4×50 ml 1N KOH.
Ether phase
Washed until neutral.
Aqueous phase D
Acidified with diluted H_2SO_4 and extracted with 4×50 ml. ether.
Aqueous phase
Discarded.
Ether phase E
Evaporated. Chromatography on Al_2O_3.
Aqueous phase
Discarded.
Ether phase
Washed with 2×10 ml. $NaHCO_3$ (saturated) and with 3×10 ml. H_2O. Evaporated to dryness. Tested for estrogens by means of paper chromatography and fluorescence.

TABLE B

Aqueous phase B (obtained in step [2] Table A) is hydrolyzed with 15 ml. HCl for 30 minutes at 100° C., then chilled and extracted with 4×50 ml. ether.

Aqueous phase
Discarded.

Ether phase
Washed with 2×20 ml. $NaHCO_3$ (saturated) and with 1×10 ml. H_2O.

Aqueous phase
Discarded.

Ether phase
Evaporated to dryness at a reduced pressure. Residue dissolved in 5 ml. ether and 54 ml. CCl_4. Extracted with 4×20 ml. KOH (1N).

Aqueous phase
Acidified with diluted H_2SO_4 and extracted with 4×50 ml. ether.

CCl_4-phase
Washed until neutral. Evaporated to dryness at a reduced pressure. Zimmermann-reaction for 17-ketosteroids with the residue.

Aqueous phase
Discarded.

Ether phase
Washed with 2×10 ml. $NaHCO_3$ (saturated) and with 3×10 ml. H_2O.

Aqueous phase
Discarded.

Ether phase
Evaporated to dryness at a reduced pressure. Tested for estrogens by means of paper chromatography and by a fluorescence method.

TABLE C

Residue C (obtained in step [1], Table A) is suspended in 200 ml. H_2O, followed by the addition of 200 ml. 5% NaOH and hydrolysis at room temperature for 48 hours. The pH is adjusted to 9 and the material is extracted with 5×150 ml. ether.

Aqueous phase
Discarded.

Ether phase
Washed with 2×20 ml. $NaHCO_3$ (saturated) and with 1×10 ml. H_2O.

Aqueous phase
Discarded.

Ether phase
Evaporated to dryness at a reduced pressure. Residue dissolved in 5 ml. ether and 54 ml. CCl_4. Extracted with 4×20 ml. KOH (1N).

Aqueous phase
Acidified with diluted H_2SO_4 and extracted with 4×50 ml. ether.

CCl_4-phase
Washed until neutral. Evaporated to dryness at a reduced pressure. Zimmermann reaction for 17-ketosteroids.

Aqueous phase
Discarded.

Ether phase
Washed with 2×10 ml. $NaHCO_3$ (saturated) and with 3×10 ml. H_2O.

Aqueous phase
Discarded.

Ether phase
Evaporated to dryness at a reduced pressure. Residue tested for estrogens by means of paper chromatography and by a fluorescence method following chromatography on Al_2O_3.

EFFECT OF CORTISONE ON SODIUM METABOLISM IN SIMMONDS' DISEASE

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THE early reports on the use of cortisone in Simmonds' disease were not entirely favorable (1, 2). These were followed by numerous case records describing substantial benefit following the use of this drug when given orally in small daily doses between 12.5 and 25.0 mg. (3–7).

Recently Whittaker and Whitehead (8) drew attention to the occurrence of water retention in anterior hypopituitarism. Later Wynn and Garrod (9) showed that cortisone was effective in correcting the abnormality.

This paper describes a series of measurements of exchangeable sodium in a case of Simmonds' disease before and after treatment with cortisone. Several measurements were made before cortisone treatment was started and they were continued for eighteen months afterwards.

METHODS

For each measurement a 70-μc. dose of Na^{24} was given intravenously, according to the technique of Miller and Wilson (10). After allowing twenty-four hours for the isotope to reach equilibrium of distribution, two 30-ml. blood samples were withdrawn and the specific activity of the serum sodium was calculated in duplicate. The urinary excretion of the isotope was measured and the exchangeable sodium determined from the formula:

$$\text{Exchangeable sodium} = \frac{\text{Radioactivity injected} - \text{radioactivity excreted}}{\text{Radioactivity per mEq. of serum sodium}}.$$

CASE HISTORY

In a female patient, now aged 50 years, the diagnosis of anterior hypopituitarism had been made six years previously. She enjoyed good health until the age of 34, when her only pregnancy, which had run a normal course up to delivery, was complicated by a severe postpartum hemorrhage.

Thereafter lactation failed and menstruation did not recommence. During the subsequent nine years she lost 12 Kg. in weight, her appetite progressively deteriorated and she became intolerant of cold weather. Her hair became dry and brittle and was seen to be falling out eight years after delivery. Mental concentration became difficult.

Between 1948 and 1953, she was seen on several occasions in the out-patient department of Sheffield Royal Infirmary. The notes record a progressive increase in the severity of her symptoms and the development of extreme pallor, loss of pubic and axillary

Received December 14, 1955.

hair and atrophy of the genitalia. She began to have recurrent attacks of nausea and vomiting, sometimes accompanied by diarrhea.

The diagnosis of Simmonds' disease was made in 1949 and treatment was instituted with intramuscular injections of testosterone propionate and desiccated thyroid orally.

In December 1952 she had the first of a series of attacks of hypoglycemic coma, and she was admitted to the hospital then, and again in April 1953. On both occasions rapid improvement followed intravenous administration of glucose and Eucortone.

The various laboratory investigations that were carried out are summarized in Table 1. They included a radioactive iodine (I^{131}) tracer test showing thyroidal uptake values in the low normal range. The serum protein-bound iodine level at forty-eight hours was low and the T index (11) just within the myxedema range. After an intramuscular injection of thyrotropic hormone, the thyroidal I^{131} uptake at twenty-four hours increased from 30 per cent to 60 per cent, a result in keeping with thyroid hypofunction subsequent to anterior pituitary failure (12).

After admission to the hospital in April 1953, several forms of treatment were tried in this patient, and serial measurements with sodium exchange were made. An implant of 100 mg. of deoxycorticosterone acetate (DCA) induced marked edema and had to be removed. Oral *l*-thyroxine sodium, 0.1 mg. daily, had to be discontinued after eight days as the patient felt ill with severe pains in her calves and hamstring muscles. She started to vomit and became drowsy as another hypoglycemic attack developed and the blood sugar level fell to 36 mg./100 ml.

After recovering from this episode, methyltestosterone by mouth made her feel sick and had to be stopped. Then a course of corticotropin (ACTH) given by intramuscular injection resulted in a severe mental disturbance.

During the period between April and December, 1953, nine measurements of sodium exchange were made. The values fluctuated widely between 1,725 mEq. and 2,355 mEq., and the serum sodium levels varied from 115 to 141 mEq. per liter. It may be seen in Figure 1 that there is a linear relationship between the exchangeable sodium measurement and the concentration of sodium the serum.

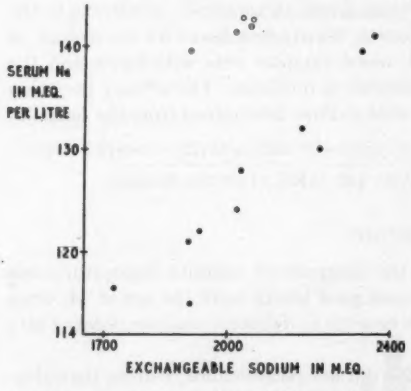

FIG. 1. Showing the relationship between the exchangeable sodium and serum sodium values prior to and during treatment with cortisone acetate, 12.5 mg. daily. The values obtained during cortisone acetate therapy are shown as open circles.

After discharge from the hospital in July 1953 the patient was observed as an outpatient, receiving no treatment. She did not feel well, all her previous complaints continued and edema developed spontaneously while her serum sodium level was 130 mEq. per liter.

She was unwilling to return to the hospital but was eventually persuaded to do so in December 1953, so that cortisone treatment could be started.

After two further measurements of exchangeable sodium, oral cortisone was started on December 3, 1953. An initial daily dose of 5 mg. of oral cortisone acetate was increased after twelve days to 12.5 mg. and has been continued at this level ever since.

TABLE 1. LABORATORY DATA IN A CASE OF SIMMONDS' DISEASE BEFORE STARTING TREATMENT WITH CORTISONE

Investigation	Result
Urinary 17-ketosteroids	0.5–3.0 mg./24 hrs. (5 observations)
Urinary 17-ketogenic steroids	1.0 mg./24 hrs.
Fasting blood glucose	36–59 mg./100 ml. (range of 10 observations)
Basal metabolic rate	−20% to −39% (4 observations)
Blood examination	Hb. 11.1 Gm./100 ml. (75%); w.b.c. 9,000/cu.mm.; differential leukocyte count, normal; blood film—normocytic and normochromic red blood corpuscles on the whole, with scattered microcytic and hypochromic cells and some macrocytes
Serum cholesterol	222 mg./100 ml. (mean of 4 readings)
Serum chloride	77–92 mEq./L.
Serum potassium	4.4–5.4 mEq./L.
Insulin sensitivity test (Dec. 12, 1952)	Blood sugar level fell to 26 mg./100 ml. after 2 units of soluble insulin intravenously
Erythrocyte sedimentation rate (Westergren)	15–39 mm./hr.
Urinary gonadotropins in 24 hrs.	No gonadotropic hormones detected at a level of 8 mouse units per liter
Electrocardiogram	Flat or inverted T waves in all leads. Low voltage in all leads
X-ray examination of skull	Pituitary fossa normal
Water diuresis test	No diuretic response to water load of 1,000 ml.
I^{131} diagnostic tracer test	4-hr. thyroidal uptake, 14% 48-hr. thyroidal uptake, 29% T index = 2.9 (11) 48-hr. total plasma activity, 0.20%/L. 48-hr. radioactive serum protein-bound I^{131}, 0.02%/L.
Thyroidal I^{131} uptake after an intramuscular injection of thyrotropic hormone (12)	Before thyrotropic hormone the 24-hr. uptake = 30% Uptake 24 hrs. after thyrotropic hormone = 60%

Her general condition improved markedly and she has enjoyed a period of fifteen months when her energy has enabled her to do her own housework. This had been too much for her for many years. Furthermore, she lost all her general symptoms and has not had any more attacks of hypoglycemia.

Five days after the start of cortisone therapy the exchangeable sodium value rose by 300 mEq. to 3,330 mEq., but five subsequent measurements up to March 1955 remained steady around the level immediately prior to receiving cortisone. The serum sodium level rose to normal and varied only between 139 and 143 mEq. per liter. These five measurements are shown as open circles in Figure 1, and are conspicuous in that they lie well above the line of the measurements made before this treatment was given. During cortisone therapy, normal serum sodium concentrations were maintained in the range of 139 to 143 mEq. per liter. Prior to this treatment there was clearly an excess of water in the body at all levels of serum sodium concentration and the administration of cortisone corrected a chronic discrepancy between the total body sodium and water content. The last measurement of sodium exchange was extended, serum samples being withdrawn at twenty-four and forty-eight hours. The values for the exchangeable sodium were 1,907 and 1,955 mEq., indicating that the period of twenty-four hours was adequate for attainment of equilibrium of distribution of the isotope under these conditions. These results are summarized in Table 2.

TABLE 2. EXCHANGEABLE SODIUM VALUES DURING VARIOUS COURSES OF TREATMENT

Date	Exchangeable sodium (mEq.)	Serum sodium (mEq./L.)	Weight (Kg.)	Treatment
Apr. 29, '53	2,180	132	46.7	Eucortone injections only
May 19, '53	1,725	116	48.1	DCA
June 9, '53	1,932	122	49.0	Thyroxine and DCA
June 16, '53	1,912	121	46.3	Just after omission of DCA
June 23, '53	2,355	141	47.7	No treatment
June 30, '53	1,908	115	47.2	Methyltestosterone
July 21, '53	2,225	130	47.6	Corticotropin (ACTH)
Nov. 17, '53	2,055	128	43.1	No treatment
Dec. 1, '53	2,025	124	43.4	No treatment
Dec. 8, '53	2,330	140	44.1	Cortisone acetate orally 5 mg. daily
Jan. 5, '54	2,068	142	44.1	Cortisone acetate orally, 12.5 mg. daily
Jan. 26, '54	2,035	143	44.8	Cortisone acetate orally, 12.5 mg. daily
Feb. 23, '54	2,056	142	47.3	Cortisone acetate orally, 12.5 mg. daily
Nov. 9, '54	2,022	141	51.5	Cortisone acetate orally, 12.5 mg. daily
Mar. 16, '55	1,907	139	49.2	Cortisone acetate orally, 12.5 mg. daily

COMMENT

Wide fluctuations in exchangeable sodium and serum sodium values occurred in this patient during 1953. Similar variations in serum sodium level were found by Sheehan and Summers (13). The mechanism of these changes is not clear, as there is evidence that the excretion of aldosterone is not controlled by adrenocorticotropic hormone (14–17) and patients with hypopituitarism have been reported to excrete normal quantities of salt-retaining hormone in the urine (15, 18).

The low levels of exchangeable sodium in our patient may have been due to prolonged extrarenal loss during attacks of vomiting. The administration of cortisone did not cause a permanent rise in exchangeable sodium, indicating that the amount of sodium available for exchange at the time cortisone was started was adequate for the maintenance of health.

It can be seen that the rise in serum sodium concentration after giving cortisone was not accompanied by any immediate alteration in weight and the exchangeable sodium, after rising initially, fell within a month to the same level as that of the two observations made immediately before starting cortisone acetate.

Previously any rise in serum sodium level to the normal range had always been accompanied by a corresponding rise in exchangeable sodium. The two lowest values for serum sodium—116 and 115 mEq. per liter—were obtained on May 19, 1953 and June 30, 1953 when the two lowest exchangeable sodium readings were obtained. At this time the patient weighed from 47 to 48 Kg., though at other times her weight fell to 43 Kg.

These measurements, and the clinical evidence of edema during August 1953, suggest that the patient was liable to excessive retention of water from time to time as described by Whittaker and Whitehead (8) and Wynn and Garrod (9).

The five observations made while the patient was taking cortisone acetate by mouth now extend over eighteen months and the constant serum sodium level and the steady exchangeable sodium contrast sharply with the data obtained during the previous eight months.

After a month of cortisone therapy, the patient began to gain weight and this continued during the subsequent eleven months. Eventually her weight rose by 5 Kg. to 50 Kg., though there was no increase in the exchangeable sodium. It is, therefore, probable that this gain was due to increase in fat or muscular tissue and not to retention of sodium and water.

Apart from the stabilization of her sodium metabolism this patient has ceased to suffer from hypoglycemic attacks which had frequently necessitated hospital treatment.

SUMMARY

Serial measurements of exchangeable sodium have been made in a case of Simmonds' disease.

Wide fluctuations in exchangeable sodium occurred during a period of eight months when various forms of treatment were introduced. During this time exchangeable sodium altered proportionately as the concentration of serum sodium varied.

This period with its wide fluctuations contrasts sharply with a subsequent period of eighteen months when the patient received oral cortisone and when both the exchangeable sodium and serum sodium remained constant.

The patient derived great benefit from the cortisone treatment and her symptoms disappeared.

Acknowledgment

I wish to acknowledge the assistance of Prof. G. M. Wilson and Dr. H. Miller. The Endowment Fund of the United Sheffield Hospitals, the Medical Research Fund of the University of Sheffield and the Sheffield Regional Hospital Board Research Fund helped with financial grants.

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THE METABOLISM OF IODOTYROSINES. III. DI-IODOTYROSINE DESHALOGENATING ACTIVITY OF HUMAN THYROID TISSUE

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DI-IODOTYROSINE is readily deiodinated by tissue slices from many organs. Hartmann (1) observed that up to 25 per cent of di-iodotyrosine was deiodinated by dog liver slices. Deshalogenation was demonstrated to lesser degree in various other tissues, including kidney and thyroid from the dog, rabbit, guinea pig and rat. These observations have been criticized for technical reasons and extended by Roche and his colleagues (2, 3) using I^{131}-labeled mono- and di-iodotyrosine as substrate. Deiodination was observed in the thyroid of the sheep and rabbit, kidney of the dog, and in other tissues. In the lung of the rabbit and dog, there was no activity. This deshalogenating enzyme has been concentrated and characterized by Roche *et al.* (3). It is specific for the halogen derivatives of tyrosine, but does not deshalogenate thyroxine or tri-iodothyronine.

From *in vivo* studies in the rat, Fawcett and Kirkwood (4) obtained evidence that the salivary gland is the principal and perhaps the only organ for the deiodination of di-iodotyrosine *in vivo*, but Tong, Potter and Chaikoff have been unable to reproduce their findings (5).

It was shown in the preceding paper of this series (6) that certain patients with congenital goiter characteristically are unable to deiodinate di-iodotyrosine when this substance is injected intravenously. It became of interest therefore, to test the deshalogenase activity of sheep and bovine thyroid tissue slices and of human pathologic thyroid tissue removed at the time of surgical thyroidectomy. This was of particular importance because one of the patients with congenital goiter (Patient B of Ref. 6) was to have a thyroidectomy for rapid growth of the gland and beginning symptoms of tracheal compression.

METHODS

Sheep and bovine thyroid glands were collected at the time of slaughter at a nearby abattoir and brought directly to the laboratory. Human glands were obtained in the

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operating theater of the hospital, and brought at once to the laboratory for sectioning.[1]

Tissues were cut as quickly as possible on a Stadie microtome into slices 0.5 mm. thick. Approximately 1-gram portions of tissue were weighed on a torsion balance after blotting, and transferred to 50-ml. Erlenmeyer flasks containing 4 ml. of Krebs-Ringer solution maintained at pH 7.4 with phosphate buffer. To each flask was added I^{131}-labeled *dl*-di-iodotyrosine (T_2*) in an amount which rendered about 30,000 counts per minute in the well-type scintillation detector. Carrier di-iodotyrosine was added in 0.2 to 1 ml. volume. Control flasks contained I^{131} or T_2* alone. Other control flasks containing tissue were heated for three minutes in boiling water before T_2* was added. The flasks were shaken at 37.5° C. for six hours while in a constant air stream. At the end of this time the flasks were either frozen in liquid air, in the deep-freeze unit, or analyzed at once. The method of analysis of tissues was that described by Roche *et al.* (2).

Labeled di-iodotyrosine was prepared by exchange (7). The *dl* isomer was used throughout. The relative concentrations of labeled di-iodotyrosine and iodide in the various preparations were determined by chromatography in a butanol-acetic acid solvent system. The analyses agreed closely with those shown in Table 1, which were obtained by extraction. From 83.0 to 90.9 per cent of the labeled iodine was present as di-iodotyrosine, and the rest as iodide.

The design of the key experiment on the gland from the patient with congenital goiter warrants special attention. On the day prior to thyroidectomy, the deshalogenase activity of a set of sheep thyroid slices was observed, using the same solutions as those to be used for the goiter of the cretin. On the morning of surgery, the gland was received on ice immediately after removal. Representative areas were taken for measurement of total labeled iodine, and for histologic study. Slices were prepared as quickly as possible. The remainder of the gland was kept on ice. Immediately thereafter, another thyroid gland from a patient with nodular goiter was received. An identical set of flasks was prepared, using slices from this gland. After six hours, the flasks containing the slices from the cretin were removed from the water bath and started through the extraction process. Immediately another complete set of slices was prepared and observed for deiodination as before. Thus the critical experiment on the thyroid from the patient with congenital goiter was bracketed with experiments on sheep thyroid slices and on tissue from a patient with nodular goiter.

The thyroid of the patient with congenital goiter was found to contain a small residual of labeled iodine from the I^{131} which had been given in the course of a study several weeks previously. Two flasks containing approximately 1 gram each of tissue were shaken as before with Krebs-Ringer phosphate solution at pH 7.4 for six hours. To another pair of flasks was added I^{131} and to another pair, T_2*. To another pair of flasks which had been heated for three minutes T_2* was also added. After six hours of shaking the tissues were ground, acidified to pH 2 with H_2SO_4, and extracted three times with *n*-butanol. Aliquots of the pooled extracts were analyzed chromatographically on Whatman #3 paper in the butanol-acetic acid solvent system (8).

RESULTS

The results of all observations are shown in Table 1. One experiment was carried out on bovine, and one on sheep thyroid tissue. Five sets of experiments were performed on human tissue received from patients with

[1] We are grateful to Dr. H. Muller for making these glands available to us.

TABLE 1. THE DEIODINATION OF I^{131}-LABELED DI-IODOTYROSINE (T_2*) BY TISSUE SLICES

The weight of samples in each flask varied from 0.85 to 1.02 Gm. With each experiment except #1, there were control flasks containing I^{131} and T_2*. Control flasks containing I^{131} yielded recoveries varying from 81.9 to 96.2 per cent. Control flasks containing T_2* yielded recoveries, as iodide, varying from 9.1 to 16.4 per cent.

Exper. No.	Date (1955)	Material	No. of flasks	Tissue condition	Carrier di-iodotyrosine	Recovery as I^{131} (% of added T_2*)
1	May 27	Beef thyroid	3	Slice	<2γ	62.7–71.3
			3	Slice, heated	<2γ	11.1–13.6
2	June 6	Nodular goiter	6	Slice	<2γ	67.6–83.8
			2	Slice, heated	<2γ	4.5– 9.6
3	June 17	Nodular goiter	3	Slice	<2γ	62.2–75.0
			2	Slice, heated	<2γ	8.6–10.3
	June 22		2	Coarse ground, frozen 5 dys.	<2γ	14.4–15.7
			1	Coarse ground, frozen 5 dys., heated	<2γ	16.0
4	June 29	Nodular goiter	2	Slice	<2γ	61.6–70.5
			2	Slice, heated	<2γ	8.9– 9.7
			2	Coarse ground	<2γ	68.3–73.0
			2	Coarse ground, heated	<2γ	9.6– 9.8
			2	Coarse ground, frozen 20 hrs.	<2γ	47.0–49.2
5	Aug. 23	Sheep thyroid	2	Slice	5γ	42.4–43.7
			2	Slice	10γ	41.4–42.5
			2	Slice, heated	5γ	12.1–13.2
6	Aug. 24	Congen. goiter	1	Slice	2γ	8.1
			2	Slice	5γ	9.3– 9.4
			2	Slice	10γ	9.3–16.3
			2	Slice	50γ	9.0– 9.5
			2	Slice, heated	10γ	13.6–13.9
7	Aug. 24	Nodular goiter	2	Slice	2γ	60.3–66.4
			2	Slice	5γ	56.6–59.1
			2	Slice	10γ	55.0–57.6
			2	Slice	50γ	53.2–53.2
			2	Slice, heated	10γ	13.9–14.9
8	Aug. 24	Congen. goiter	2	Slice	2γ	9.9–12.1
			2	Slice	5γ	9.5–11.1
			2	Slice	10γ	9.6–10.6
			2	Slice	50γ	9.9–10.7
			2	Slice, heated	10γ	14.7–15.2
9	Oct. 14	Thyroid (treated Graves' disease)	2	Slice	5γ	68.8–72.2
			1	Slice	50γ	69.1
			2	Slice	500γ	64.3–65.6
			2	Slice, heated	50γ	3.7– 4.2
10	Oct. 14	Multinodular goiter	2	Slice	5γ	34.9–65.6
			2	Slice	50γ	67.2–67.7
			2	Slice	500γ	56.4–59.2
			1	Slice, heated	50γ	3.3

nodular goiter. All these glands showed the nodularity and large colloid-filled follicles which are characteristic of the disease. One experiment involved a gland from a patient who had been treated preoperatively for Graves' disease with propylthiouracil. Two sets of experiments were performed on the thyroid removed from the patient with congenital goiter.

Deshalogenase activity was demonstrable in both sheep and bovine thyroid slices. Deshalogenation in the sheep slices was the same following addition of either 5 or 10 micrograms of di-iodotyrosine as substrate. There was no more labeled iodide recovered from the flasks containing heated slices than could be accounted for by the labeled iodide which was present in the preparation of labeled di-iodotyrosine added as the substrate.

All tissue slices from nodular goiters, and those from the subject with treated Graves' disease, showed deiodinating activity. In most cases more than 50 per cent of the labeled substrate was recovered as iodide, and only a single flask (Experiment #10) showed a recovery of less than 50 per cent as iodide. Part of the gland used in Experiment #3 was ground coarsely and frozen for five days before a repeated attempt was made to demonstrate deshalogenation. There was no evidence of any deiodination. Ground tissue was compared to slices in Experiment #4. There appeared to be no difference in deshalogenating activity, but when the ground tissue had been frozen for twenty hours, there was less deiodination of substrate than before.

The quantity of substrate di-iodotyrosine was varied in the last three experiments from 5 to 500 micrograms. Only in Experiment #7 was there a fall in the fraction of the substrate which was deiodinated as the concentration of substrate was increased.

Thyroid tissue slices from the patient with congenital goiter failed to deiodinate di-iodotyrosine (Experiments #6 and #8). Indeed, it appeared that slightly less labeled iodine was recovered as iodide than from heated slices, or from flasks containing labeled substrate alone. This finding was consistent with utilization by the slices of part of the iodide which was added with the labeled di-iodotyrosine.

Acid butanol extracts of slices from the patient with congenital goiter when analyzed chromatographically showed no evidence of any iodinated substance, in spite of the fact that there was residual labeled iodine in the gland from an earlier experiment. This labeled iodine was apparently firmly bound to protein. There was no chromatographic evidence of any acid butanol-extractable substance other than iodide after incubation of these tissues for six hours with I^{131}. Chromatographic analysis of acid butanol extracts of tissue after six hours of incubation with T_2* disclosed small amounts of labeled iodine which ran on the paper strips with the latter part and just beyond the zone corresponding to tri-iodothyronine and

thyroxine. In position this area was identical to a zone of activity seen in in the blood and urine of this patient after injection of I^{131} and T_2*. Almost all of the labeled iodine was present as T_2*.

Histologic study of the specimen of thyroid removed from the goitrous cretin demonstrated intense hyperplasia identical to that seen in the patient's brother (9).

DISCUSSION

The findings reported here on the deshalogenase activity of sheep, bovine, and pathologic human thyroid tissue are in keeping with those of Roche (2, 3). Both normal animal and certain pathologic human thyroid tissues readily perform the deshalogenation of di-iodotyrosine. Within the limits of the experimental design of these studies, striking differences were not observed among the various specimens of nodular goiter available for study. Deshalogenation was completely inhibited by heating the tissue. Variation in quantity of substrate from less than 2 micrograms to 500 micrograms appeared to make little difference in the fraction of the substrate deiodinated. Thus, the findings indicate that the enzyme was present in excess, even when 500 micrograms was the amount of substrate per gram of tissue. The potency of the deshalogenating system has been alluded to by others (10) in studies in which deiodination took place even when large amounts of substrate were either ingested, injected, or added to tissue *in vitro* (1).

Failure of deshalogenation of di-iodotyrosine by tissue slices from the thyroid of the cretin with congenital goiter was confirmatory of *in vivo* studies of the fate of injected labeled di-iodotyrosine in the brother of the patient. Subsequent to thyroidectomy this patient was also found to be unable to deiodinate injected di-iodotyrosine. *Thus the thyroid gland of this patient, and presumably that of other similar patients, shared with the rest of the body an inability to deiodinate di-iodotyrosine.*

It has been suggested by Gross *et al.* (8) and emphasized by Roche *et al.* (2) that there is a recycling of iodide within the thyroid gland. In this scheme mono- and di-iodotyrosine are mobilized along with tri-iodothyronine and thyroxine in the process of hormone release, but T_1 and T_2 are deiodinated prior to release from the gland and, therefore, fail to reach the blood. One of the degradation products, iodide, is re-utilized in the formation of hormone and hormone precursors.

The gland of the patient with congenital goiter described here (and probably those of 2 patients with congenital goiter previously described) evidently was unable to deiodinate di-iodotyrosine, and presumably monoiodotyrosine as well. Hormone precursors were thereby lost from the gland. Since deiodination could not take place elsewhere, as in the kidney, these

substances were lost from the body. Thus a situation of extreme iodine-want developed, resulting in an intensely hyperplastic gland devoted primarily, if not exclusively, to the synthesis and secretion of the earliest substances in the synthetic chain of thyroid hormone.

SUMMARY

1. Deshalogenation of I^{131}-labeled di-iodotyrosine was demonstrated in sheep and bovine thyroid tissue slices, and in thyroid tissue from 5 patients with nodular goiter and 1 with treated Graves' disease.

2. Thyroid tissue slices from a patient with familial goitrous cretinism failed to deshalogenate labeled di-iodotyrosine.

3. The metabolic disorder of the patient with familial cretinism and that of similar patients is conceived as a general somatic failure of the normal process of deshalogenation of mono- and di-iodotyrosine. The hypothyroid state and the hyperplastic goiter are presumed to result from the loss of hormone precursors from the thyroid and from the body.

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TECHNICAL CONSIDERATIONS IN I^{131} TRACER STUDIES

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THE use of radioactive iodine as a diagnostic agent has become a well accepted procedure, often regarded as providing the most exact single test of thyroid function. Medical literature contains descriptions of many widely variable techniques, and it would appear that none is perfect or universally accepted.

There are three basic concepts of the utilization of I^{131} as a measure of thyroid function. One of these is the amount of available iodine that is acquired by the thyroid gland—and we may also observe the rate of accretion (1, 2). A second correlative study is the amount and rate of urinary excretion of iodine (1, 3–7). The third concept is the measure of protein-bound iodine in the circulating blood, indicative of the production of thyroxine (8–11).

It occurred to us that it would be worth while to perform several advocated tests with I^{131} upon one specific group of patients, in order to evaluate them for our own use. This report embodies such observations over a period of three and a half years.

METHODS AND MATERIAL

Methods

According to the technique of Corrigan and Hayden (12), a standard dose of 50 microcuries of radioactive iodine was given by mouth. Over a period of twenty-four hours, contact counts were made at two-hour to four-hour intervals over each lobe and the isthmus of the thyroid gland, using a Geiger-Mueller end-window counter. The sum of these counts recorded and plotted against time indicated the rate and degree of thyroidal uptake, to be compared with the average normal value. During the same interval, separately voided specimens of urine were assayed for radioactivity and the results were similarly plotted.

The plasma clearance test of Berson *et al.* (13) was only partly utilized for comparative purposes. Using a bismuth-wall, side-window tube at a distance of 30 centimeters, total thyroidal uptake counts were made very alternate minute for thirty minutes following the intravenous injection of a standard tracer dose of I^{131}. The counts were plotted against time, the slope of the successive counts indicating thyroid activity. In Figure 3 the rate of increase is expressed as the percentile increase of the thirty-minute count over the two-minute count after intravenous injection.

Received December 10, 1955.

Counts of thyroidal uptake were also obtained with a bismuth-wall tube at a 30-centimeter distance at intervals of two, four, eight, and twenty-four hours following the intravenous administration of 50 to 100 microcuries of I^{131}. The administered dose had previously been counted at the same distance with the same tube. A similar procedure has been described by Kriss (14).

The actual status of thyroid function was determined by the clinical history, physical findings, customary laboratory procedures, and occasionally by response to therapeutic measures. The subsequent clinical courses were followed by means of questionnaire information from the referring physicians. In all cases the follow-up study continued for at least six months after the last diagnostic test with radioactive iodine.

Material

Three hundred and sixty-four consecutive tracer studies were performed upon 302 patients (16 per cent male) at the Deaconess Hospital. The age of the youngest patient was 18 months, and that of the oldest was 83 years; 72 per cent were over 35 years of age. Forty-three patients (14 per cent) had diffuse thyroid enlargement with exophthalmos, and 90 (30 per cent) had diffuse thyroid enlargement without exophthalmos. There were 13 patients with nodular goiter, of whom 3 exhibited exophthalmos, and there were 19 patients with a solitary thyroid nodule. Substernal goiter was indicated roentgenographically in 6 cases. Twenty-five patients had received some sort of hormone therapy prior to the tracer study, and 52 had had propylthiouracil or one of the other antithyroid drugs. Twenty-four other patients had been treated with stable iodine, and 41 had had a thyroidectomy. Classification of cases as to thyroid function, clinically determined, is shown in Table 1.

RESULTS

The method of charting is based on the theory of probability. The total period of time covered by the study (whether it be with regard to percentage uptake, urinary excretion or counts per minute) is divided into

TABLE 1. STATUS OF THYROID FUNCTION IN 302 PATIENTS ON WHOM 364 TRACER STUDIES WERE PERFORMED

		No. of cases
1. Hyperthyroidism		107
2. Euthyroidism		182
A. Primary cardiac disease	13	
B. Legg-Perthes disease	1	
C. Thyroid carcinoma	4	
3. Hypothyroidism		13
A. Thyroid carcinoma	2	
B. Primary cardiac disease	1	
Total		132

ten equal intervals. The number of observations falling within any interval is plotted against the percentage. The graphs therefore represent distribution.

Of 186 cases studied by Geiger-Mueller contact counting, the ultimate clinical diagnoses were 81 cases of hyperthyroidism, 93 of euthyroidism, and 12 of hypothyroidism. The count distribution is shown in Figure 1. Hypothyroidism is omitted, as this condition is not distinguishable by counting in this manner. The euthyroid patients show a sharply pronounced peak at 2,000 to 3,000 counts per minute; the hyperthyroid patients show a peak at 10,000 to 15,000 counts per minute. However, both groups are scattered over the entire range, so that there is no clear distinction between them by this method, and the definite overlap limits the usefulness of this method. At the level of 2,000 to 3,000 counts per minute, there is an 85 per cent certainty of euthyroidism; and between 3,000 and 4,000 counts per minute there is still a 70 per cent chance of euthyroidism, so far as the tracer study is concerned.

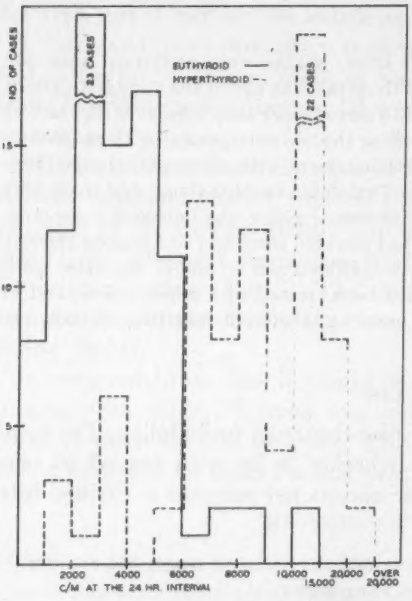

FIG. 1. The 24-hour thyroidal I^{131} uptake in 186 cases studied by means of Geiger-Mueller counting at contact over the pyramidal lobes and isthmus of the thyroid gland, after the oral administration of 50 microcuries of I^{131}. The overlap of hyperthyroid and euthyroid cases is considerable, and places definite limitations on the method. The hypothyroid group was completely overlapped by the other groups and is not included on the graph.

Excretory studies carried out on 126 hyperthyroid and 202 euthyroid patients are tabulated in Figure 2. The zone of overlap is in the area representing from 20 to 60 per cent excretion of the administered dose, and practically invalidates any diagnostic conclusion from this method alone for 194 patients.

The thirty-minute thyroidal uptake studies are recorded in Figure 3. The graph represents the percentage increase in the count over the thyroid at thirty minutes as compared with the count obtained at two minutes after intravenous administration of the dose of I^{131}. Again, the overlap is seen to be scattered over almost the entire range, and it appears that this method is lacking in diagnostic discrimination.

TABLE 2. THYROIDAL I^{131} UPTAKE COUNTS AT VARIOUS TIMES AFTER ADMINISTRATION OF I^{131}, IN VARIOUS THYROID STATES

Counts were made by means of a bismuth-wall tube at 30 cm. distance and expressed as percentage of the dose of I^{131} administered intravenously. The counts made at 2, 4, 8, and 24 hours after administration all show superior discrimination in comparison to the previously described methods. Of the four time-intervals, the 4-hour and 8-hour counts were the most satisfactory as a measure of thyroid function.

Diagnosis	Thyroidal uptakes of I^{131} (% of dose)									
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
2-Hour										
Euthyroid	0	21	87	30	2	0	0	0	0	0
Hyperthyroid	0	0	5	14	14	20	6	4	5	1
Hypothyroid	0	4	1	0	0	0	0	0	0	0
4-Hour										
Euthyroid	1	27	83	30	0	0	0	0	0	0
Hyperthyroid	0	0	2	7	11	17	11	9	7	2
Hypothyroid	0	5	0	0	0	0	0	0	0	0
8-Hour										
Euthyroid	1	35	71	30	2	0	0	0	0	0
Hyperthyroid	0	0	1	5	10	17	11	10	6	3
Hypothyroid	1	3	0	0	0	0	0	0	0	0
24-Hour										
Euthyroid	7	33	42	37	10	0	0	0	0	0
Hyperthyroid	0	0	2	6	11	15	18	13	9	0
Hypothyroid	3	1	0	0	0	0	0	0	0	0

Table 2 represents counts over the thyroid expressed as percentage of the administered dose obtained at intervals of two, four, eight, and twenty-four hours after intravenous injection of 50 to 100 microcuries of I^{131}. It has not been represented graphically because the tabulation shows clearly what would have otherwise required four graphs. The following conclusions may be drawn. 1) At no counting period is there any doubt about the diagnosis if the uptake exceeds 50 per cent. 2) The zone of diagnostic uncertainty (discriminating between hyperthyroidism and euthyroidism) is limited to the thyroidal uptake counts in the range of 30 to 50 per cent. The degree of uncertainty is much less for counts made at four-hour and eight-hour intervals as compared with either shorter or longer periods of time. In some of these patients the uptake was modified by medication or surgery. 3) At all intervals the method is superior to the other methods described as indices of thyroid function.

DISCUSSION

The methods described here for the estimation of thyroid function, using

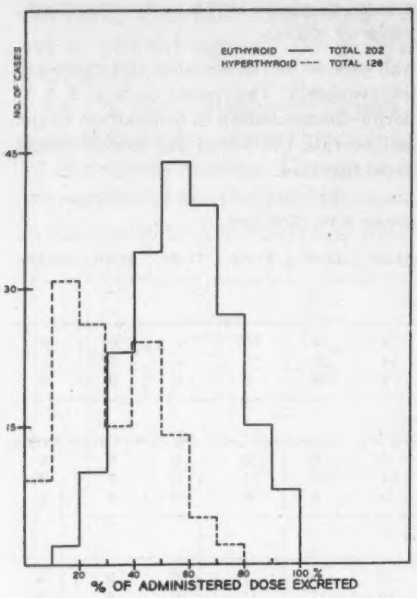

FIG. 2. The 24-hour urinary excretion of I^{131} in 328 cases. Of these, 194 cases fall in the overlap zone, making the method almost without value.

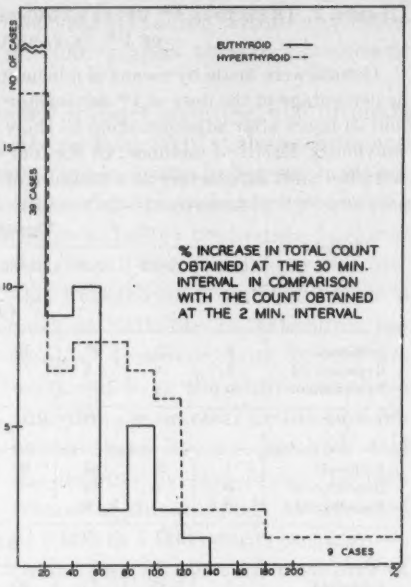
EUTHYROID ——
HYPERTHYROID --
NO. OF CASES
15
10
5
39 CASES
% INCREASE IN TOTAL COUNT OBTAINED AT THE 30 MIN. INTERVAL IN COMPARISON WITH THE COUNT OBTAINED AT THE 2 MIN. INTERVAL
9 CASES
20 40 60 80 100 120 140 160 180 200
%

FIG. 3. Half-hour thyroidal I^{131} uptake studies after the intravenous administration of 50 to 100 microcuries of I^{131}. There is practically no difference between euthyroid and hyperthyroid patients.

radioactive iodine, have been advocated by various investigators. Our own techniques have been reasonably exact, though not always identical with those originally described. It has been our purpose to make a comparative study in order to test reliability and technical simplicity.

Contact counting over the thyroid gland with a Geiger-Mueller end-window tube, held in the hand, would seem to have merit in detecting areas of the thyroid gland in which function is excessive, diminished, or absent. Since counts are made at the point of contact with the skin, and only part of the gland is counted at one time, the total thyroid count is only an approximation. It logically follows that such inaccuracy will result in a greater number of indeterminate cases falling in the range of difficult diagnoses. Our observations clearly demonstrate this inconclusiveness.

Urinary excretion of I^{131} was one of the earlier advocated methods for determination of thyroid function. Our observations were made upon separately voided urine specimens during a 24-hour period. The results were not sufficiently conclusive, either as to rate or total amount of excretion, to make them of diagnostic value.

The half-hour thyroidal increment test, adapted from Berson's method

of determining the rate of plasma clearance, was found to be not sufficiently discriminative. The early accretion of iodine by the thyroid can be too easily modified by circumstances that are within the range of normal physiology. It seems likely that the availability of stable iodine would have a modifying effect upon the rate of thyroid acquisition. However, this technique introduces counting with a bismuth-wall tube at a distance which allows the use of only gamma emission, thus including the total thyroid at one time. This seems to offer the technical advantage of precision.

The counting method of our choice, the reliability of which has been represented in Table 2, is advocated, not only because of its superior discriminative value, but also because of technical simplicity. The factors influencing absorption are reduced to basic terms by intravenous administration. Physical comparison involves only substitution of the thyroid gland for a flask as the I^{131} container, with no change in distance or quality of radiation. Geometric error is greatly reduced by the selection of the relatively great distance of 30 centimeters. Moreover, the method requires no more equipment than is available in any isotope laboratory.

Our observations lead us to believe that the empirically selected time of twenty-four hours after administration of the dose of I^{131} is not a logical choice for diagnostic reliability. Rather, it would appear that counts made at four and eight hours are more dependable and contribute to the convenience of the patient.

It is not our purpose to offer counting of thyroidal uptake of I^{131} as the ultimate laboratory procedure in estimating thyroid function. At present, we prefer it because of its reliability and simplicity.

SUMMARY AND CONCLUSIONS

1. Three hundred and sixty-four tracer studies performed on 302 patients are reported in order to demonstrate the comparative value of several I^{131} thyroidal uptake and urinary excretion techniques.

2. The most accurate I^{131} method for the determination of thyroid function consists of counting with a bismuth-wall tube at a distance of 30 centimeters from the radiating source.

3. Intravenous administration of the tracer dose eliminates technical inaccuracy arising from variable rates of absorption from the gastrointestinal tract.

4. Counting intervals of four and eight hours offer advantages of greater diagnostic discrimination and convenience over the commonly employed 24-hour interval.

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GOITER AND MYXEDEMA FOLLOWING PROLONGED INGESTION OF IODINE: REPORT OF A CASE WITH EVIDENCE OF UNUSUAL THYROID DYSFUNCTION

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ATTENTION has been directed recently to the occasional role of intrinsic defects in the thyroid mechanism for the conversion of inorganic iodide to thyroid hormone as a cause of goiter and hypothyroidism (1–9). The possible role of chronic or excessive iodine administration in the production of goiter and hypothyroidism has also been emphasized in several clinical reports (10–14). The case here described presents features compatible with both these causes of thyroid dysfunction and enlargement.

CASE REPORT

A.H. (H.U.P. #033665): This 52-year-old man was first seen in June 1954, complaining that after swallowing a bolus of food he had a heavy sensation in the epigastrium, which was relieved by drinking liquids; this had been present intermittently for several years. Other symptoms included increasing fatiguability and huskiness of the voice for the previous year and, more recently, generalized myalgia, weakness of the legs, sensitivity to cold, dryness of the skin, loss of body hair, a moderate gain in weight, and puffiness of the hands and face. There had been recurrent symptoms of respiratory allergy since the age of 12, treated by various medications, several attempts at hyposensitization and removal of nasal polyps. For twelve to fifteen years he had been taking, three to four times daily, an "asthma" preparation containing 30 mg. of iodopyrine (4.04 per cent iodine by weight), in addition to aminopyrine, lobeline sulfate and caffeine.

He was 66½ inches tall and weighed 168 pounds. Blood pressure was 120/75, and pulse rate 60 per minute. His voice was hoarse and croaking, the skin was dry and sallow, and there was periorbital puffiness and slight pretibial edema. His hair was thin and dry on the scalp, missing on the outer third of the eyebrows and absent or sparse in the axillae and over the thorax, arms, abdomen and pubic region. The tongue was somewhat large and thick, but normally papillated. A diffuse, irregular thyroid enlargement of moderately firm consistency was palpable on swallowing, but could not be completely outlined since it appeared to extend into the upper thorax. The external genitalia were normal. All of the tendon reflexes, the achilles in particular, exhibited a slow relaxation phase. Laboratory studies immediately after withdrawal of all medication revealed the following:

Received December 7, 1955.

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The hemoglobin level was 11.7 Gm. per 100 ml., and the white blood cell count was normal. Routine urinalyses showed no formed elements, protein or sugar. The BMR was −28 per cent. The total serum cholesterol level was 273 mg. per 100 ml. The serum protein-bound iodine level was 6.3 μg. per 100 ml. before, and 4.4 μg. per 100 ml. twenty-four hours following injection of 10 units of thyrotopin intramuscularly (normal range in this laboratory, 4.1−6.0 μg. per 100 ml.). An oral glucose tolerance test showed a blood sugar curve with fasting, half-hour, one-hour and two-hour values of 76, 148, 117 and 86 mg. per 100 ml., respectively. Serum sodium, potassium, chloride, calcium, inorganic phosphorus and total protein levels, A/G ratio, and blood urea nitrogen concentration were all within normal limits. A total circulating eosinophil count of 162 per cu. mm. fell to 16 per cu.mm. four hours following the intramuscular injection of 25 units of an aqueous preparation of corticotropin (ACTH). Urinary 17-ketosteroid excretion was 5.6 mg. per twenty-four hours, and the urinary titers of gonadotropin and estrogen were within the normal limits for an adult male. X-ray examination of the chest revealed a cervical mass extending into the upper anterior mediastinum (on a subsequent admission, automatic scanning with a scintillation counter following a tracer dose of I^{131} showed uniform radioactivity over this same general area); there was no cardiac enlargement. The sella turica was normal by x-ray examination. Electrocardiograms showed sinus bradycardia, moderately low voltage, and diphasic to inverted T waves in all left ventricular precordial leads. The oral administration of carrier-free radioactive iodine thirteen days after the withdrawal of the "asthma" mixture resulted in thyroidal I^{131} uptake values (corrected for physical decay) of 29 per cent at one hour, 31 per cent at two hours, 35 per cent at three hours, 32 per cent at six hours, 18 per cent at twenty-four hours and 8 per cent at forty-eight hours.

Evaluation sixty-three days after discontinuation of all exogenous iodine showed that the patient had enjoyed gradually increasing energy, had lost 8 pounds in weight, and was not experiencing any difficulty in swallowing. There was new hair growth on both forearms and less periorbital and acral puffiness. The skin texture and quality of his voice were still suggestive of hypothyroidism, however. The tendon reflexes had a more normal relaxation phase. The BMR was −12 per cent, the serum cholesterol level 235 mg. per 100 ml. and the serum protein-bound iodine level 7.2 μg. per 100 ml. The electrocardiogram and chest roentgenogram were essentially unchanged. Another oral tracer dose of I^{131} resulted in an uptake and "turnover" curve similar to the original in every respect.

A serum protein-bound iodine level of 4.0 μg. per 100 ml. one hundred and thirty-three days following the initial studies contrasted sharply with a serum butanol-extractable iodine value of 0.6 μg, per 100 ml. (normal range, 3.2−6.4 μg. per 100 ml.).[1]

There was progressive but incomplete disappearance of the signs and symptoms of hypothyroidism until thyroid function was again tested two hundred and seventy-five days after withdrawal of the "asthma" powders. The BMR was +7 per cent and the serum cholesterol level 282 mg. per 100 ml. The serum protein-bound iodine and butanol-extractable iodine levels were 4.8 and 1.1 μg. per 100 ml., respectively. These values were unchanged following the administration of 10 units of thyrotropin on each of three successive days. In addition, tracer doses of I^{131} were given intravenously before and after stimulation with thyrotropin. The data relating to thyroidal uptake and plasma I^{131} levels are presented in Table 1.

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[1] Courtesy of Dr. Evelyn B. Man, Yale University School of Medicine, New Haven, Conn.

Upon completion of the foregoing studies, treatment with *l*-triiodothyronine was started. The initial dosage of 25 μg. daily was increased to 137.5 μg. daily within a period of fifty-six days without any untoward effects, and was maintained at that level for an additional one hundred days. During this interval there was definite improvement in skin texture, increased hair growth in the axillae and over the forearms, a loss of an additional 7 pounds of weight and disappearance of all periorbital puffiness. The tendon reflexes were brisk. The size of the substernal goiter was unchanged at the termination of

TABLE 1. THYROIDAL UPTAKE OF I^{131} AND PLASMA CONCENTRATIONS BEFORE AND AFTER ADMINISTRATION OF THYROTROPIN*

Time of sample (hrs. after I^{131} admin.)	Before thyrotropin			After thyrotropin		
	Thyroidal I^{131} uptake (% of dose)	Percentage of I^{131} per liter of plasma		Thyroidal I^{131} uptake (% of dose)	Percentage of I^{131} per liter of plasma	
		Total plasma iodine	Protein-bound iodine		Total plasma iodine	Protein-bound iodine
1/4	37.0	2.20	0.019	37.0	2.23	0.017
3/4	41.5	1.55	0.019	39.0	—	—
1	41.5	—	—	40.5	—	—
2	39.6	—	—	43.0	1.38	0.016
3	39.2	—	—	42.5	—	—
4	38.9	1.05	0.016	42.2	—	—
6	36.9	0.93	0.014	40.2	—	—
12	27.1	—	—	30.0	0.94	0.016
24	22.7	—	—	20.1	0.58	0.016
48	9.6	0.27	0.003	10.3	0.26	0.010
72	—	—	—	5.1	0.11	0.012

* All values corrected for physical decay.

the one hundred and fifty-six days of replacement therapy, but there were conspicuous changes in the electrocardiogram, so that it now was interpreted as being well within normal limits. The serum cholesterol level had dropped to 164 mg. per 100 ml., and the BMR was −3 per cent. The urinary excretion of 17-ketosteroids was in the low normal range—9.1 mg. per twenty-four hours.

The *l*-triiodothyronine was then withdrawn for sixty days. At the termination of this period the patient had gained 6 pounds and the clinical picture of myxedema had obviously recurred, but again without any apparent change in the size of the goiter. The BMR was −10 per cent, the serum cholesterol level 302 mg. per 100 ml., and the serum protein-bound iodine level 6.0 μg. per 100 ml. Another tracer study with radioactive iodine demonstrated thyroidal uptake and plasma concentration similar to those observed on all previous occasions.

Desiccated thyroid was subsequently utilized as replacement therapy, and the degree of subjective and objective improvement duplicated that seen following the use of *l*-triiodothyronine.

COMMENT

This patient, who had ingested 3 to 5 mg. of iodine daily for at least twelve years, presented a goiter and a picture of hypothyroidism which was atypical with respect to certain indices of thyroid function usually found in primary hypothyroidism: 1) The serum protein-bound iodine level was at no time below the lower limit of the normal range established for our laboratory, even two hundred and seventy-five days following the withdrawal of all known exogenous sources of iodine, although the serum butanol-extractable iodine values were consistently subnormal. 2) The rate of accumulation of I^{131} by the thyroid was strikingly elevated, reached a maxi-

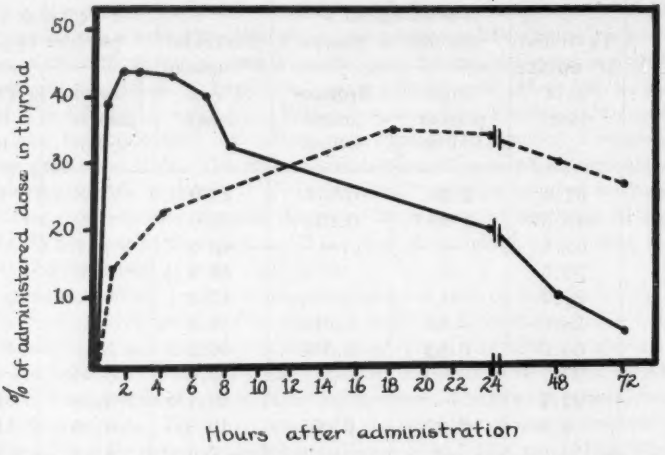

FIG. 1. Uptake of I^{131} by the thyroid gland of Patient A. H., one hundred and eighty-eight days following withdrawal of exogenous iodine (solid line), contrasted with the average values of a euthyroid individual (dotted line).

mum value in an abnormally short period of time (one to three hours), thereafter fell rapidly to a low normal value at twenty-four hours, and declined to a definitely subnormal level at forty-eight hours (Fig. 1). A corollary was the increased thyroid-plasma clearance rate of radioiodine (165 ml. per minute), comparable to that seen in thyrotoxic individuals (normal, 8–40 ml. per minute) (15).

The findings in this case appear compatible with two hypotheses, the first of which appears the more probable one:

1. *There may have been a congenital or acquired intrinsic defect in the conversion of inorganic iodide to normal thyroid hormone at some undetermined stage.* This permitted only the synthesis or release of suboptimal quantities of physiologically active hormone, which were sufficient, however, to pre-

vent overt clinical myxedema. The chronic ingestion of iodine and the consequent high serum concentration probably reduced the organic binding of iodide and the elaboration of the already limited amounts of active hormone to a level at which hypothyroidism became manifest. After withdrawal of the exogenous source of iodine, thyroid function reverted toward its previous masked suboptimal state, which could still be recognized by the persistence of the abnormal capacity to accumulate radioiodide rapidly and the low serum butanol-extractable iodine concentration.

The findings of recent studies have supported the concept that hypothyroidism and goiter may result occasionally from congenital or acquired defects and lead to the formation of inadequate amounts of normal hormone (5, 7, 16), to the production of some type of abnormal and physiologically ineffective iodinated protein complex (6–9), to a defect in the release of stored hormone (1), or possibly to an inability to deiodinate thyroxine to triiodothyronine peripherally (5, 17). The data in this case indicate a rapid accumulation but brief retention of I^{131} by the thyroid gland, occurring along with a rapidly declining concentration of inorganic radioiodide in the serum. The PBI^{131} values noted immediately after administration of the tracer dose (Table 1) were most likely due to contamination with radioiodide of the protein precipitated by trichloracetic acid rather than to any appreciable organic binding, since the plasma concentration of bound activity dropped off at approximately the same rate as the total serum values and subsequently did not rise again until after many hours, if at all. The chemical nature of the substance or substances contributing to this low order of PBI^{131} activity is unknown, since unfortunately, chromatographic studies to identify this material would have required a tracer dose of radioiodide of considerably greater activity than was thought advisable to administer. The finding of a subnormal butanol-extractable iodine level in conjunction with a normal serum protein-bound iodine value nine months after the withdrawal of exogenous iodine suggests that some abnormal, and probably inactive, iodinated protein complex contributed disproportionately to the total serum protein-bound iodine. That this patient was able to respond satisfactorily to the dosage of desiccated thyroid usually utilized for replacement therapy in hypothyroidism also indicates that this hypothyroid state was due to a deficiency of endogenous thyroxine, rather than to an inability to utilize thyroxine.

Experimental evidence suggests that the intake of excessive amounts of inorganic iodide may affect thyroid function in one or both of the following ways: a) by blocking, at least temporarily, the organic binding of elemental iodine, and b) by suppressing the release of stored thyroid hormone. Whether these effects are produced at the thyroid level by the reaction of ele-

mental iodine with inorganic iodide to form an inert molecular compound (triiodide) (18–20), or by inactivation of thyrotropin (21, 22), remains uncertain. The literature contains several case reports of goiter, hypothyroidism, or both observed during or after the administration of compounds containing iodine (10–14). The evidence indicating a superimposed effect of exogenous iodine in this patient consists of his subjective improvement, loss of weight, increased growth of body hair, normal achilles tendon reflexes, disappearance of the electrocardiographic abnormalities, loss of his myxedematous aspect and a rise in the BMR from −28 to +7 per cent within nine months after the withdrawal of the "asthma" preparation containing iodopyrine, and prior to the administration of *l*-triiodothyronine. We have considered the possibility that the aminopyrine itself may have had some goitrogenic action, but have been unable to find any specific reference to such a property of this compound, although its structure resembles that of other known goitrogens.

2. *The prolonged ingestion of excessive amounts of iodine may have produced certain sequential effects upon a previously normal pituitary-thyroid mechanism.* These effects may have been: a) impairment of normal organic binding of iodine and synthesis of thyroid hormone, and/or its subsequent release; b) compensatory hyperplasia or hypertrophy of the thyroid gland consequent to increased thyrotropin activity, with persistent goiter, although recent data cast doubt on the validity of this servo-mechanism concept of the thyroid-pituitary relationship (23–25); and c) persistence of these acquired abnormalities at least nine months following the withdrawal of the iodine preparation. Three of the reported cases of apparent "iodic" goiter and myxedema demonstrated an I^{131} uptake curve comparable to that of our patient during the period of iodine administration or within several days after withdrawal (11, 12). This hypothesis leaves unexplained the unusual difference between the serum butanol-extractable and protein-bound iodine levels in our patient nine months following elimination of the medication. Furthermore, we are unaware of any evidence that protracted alterations in thyroid function may follow the withdrawal of exogencus iodine after prolonged or excessive administration, with the possible exception of the more permanent remissions of Graves' disease resulting from iodine therapy.

The possibility that "permissive" minimal concentrations of thyroid hormone are necessary for optimal function of the thyroid mechanism, and that suppression of this essential concentration by the protracted action of excessive iodine may give rise to myxedematous changes in the thyroid itself, offers an intriguing field for speculation. However, the persistence of the original pattern of thyroid dysfunction in this patient sixty days after the termination of an extended period of treatment with *l*-triiodothyronine is not compatible with this concept.

SUMMARY

A case is described in which goiter and myxedema occurred in a 52-year-old man who had ingested 3 to 5 mg. of iodine daily for approximately twelve years. Studies demonstrated excessively rapid accumulation and discharge of radioiodide by the thyroid, evidence of only minimal thyroidal organic binding of I^{131} and an abnormally elevated non-butanol-extractable component in the serum protein-bound iodine. Incomplete subjective and objective improvement followed the withdrawal of exogenous iodine, although the intrinsic aberrations of iodine metabolism persisted along with the elevated level of the non-butanol-extractable protein-bound iodine fraction. The administration of *l*-triiodothyronine for one hundred and fifty-six days induced a euthyroid state, but no change in the size of the goiter. Re-evaluation of this patient's thyroid function following a treatment-free period showed persistence of the abnormalities noted during the original isotope studies.

Two hypotheses compatible with the observed data are discussed. These include the possibility of an intrinsic metabolic defect in the thyroid gland complicated by the effect of protracted iodine administration, and that of a sustained alteration of thyroid function perhaps entirely due to the excessive ingestion of iodine. The first of these hypotheses would appear to be the more likely one.

Acknowledgments

The authors are indebted to Mr. Robert Gorson for his advice and interest in this study, and to Miss Sandra Betz for her technical assistance. The *l*-triiodothyronine was generously supplied by Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

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Letters to the Editor

GENETIC SEX: CHROMATIN TEST VERSUS GONADAL HISTOLOGY

To the Editor

Recently we reported 5 cases of Klinefelter's syndrome in which examination of the oral smears revealed that the somatic cells contained the nuclear chromatin masses characteristically found in normal females (1). Since that time one scrotal gonad was removed from each of 3 patients in this series. Several blocks were cut from each gonad and the usual histologic pattern of clumped "interstitial cells," sclerosed tubules and tubules containing only "sustentacular cells" was seen. In some blocks from each gonad, a few isolated tubules exhibited gametogenesis of the male type. Figure 1 is a high-power view of such a tubule. Thus, the histologic evidence in each gonad was apparently contradictory to the chromatin finding in the somatic cells.

In the past, the sex of an individual has been based on the morphology and histology of the gonads. Barr and his co-workers (2, 3) showed a nuclear chromatin pattern in somatic cells which correlates with the sex of an individual. However, in a recent article (4) Barr pointed out that the true significance and the morphologic identity of the "sex chromatin" is unknown, and he makes the statement: "In particular, it is premature to equate female-type nuclei with genetic femaleness or male-type nuclei with genetic maleness, as a generalization in errors of sex development, for our present methods give no direct information concerning the genes that are concerned with sex determination and sex differentiation."

The warning was published after several investigators had accepted the male-type nuclei as proof that many patients with Turner's syndrome were actually genetic males (5, 6, 7). This belief was based on the correlation with Jost's experimental demonstration (8) that when male rabbit embryos were castrated early in gestation, they were born as apparent females. The only evidence of maleness in the cases of Turner's syndrome was the result of the chromatin test. In these patients, the absence of gonads fails to provide the usual histologic evidence of the validity of the chromatin findings.

In our 3 cases of Klinefelter's syndrome, the presence of male-type gametogenesis is an apparent contradiction of the presence of female-type

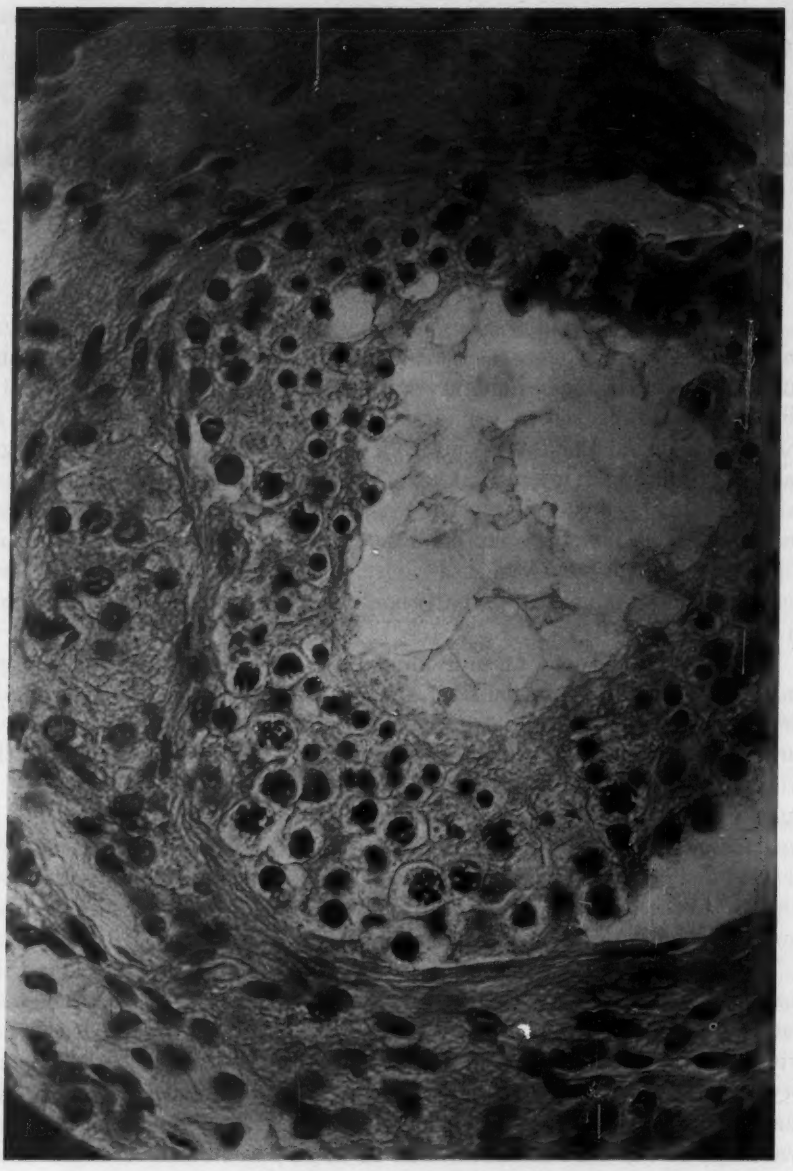

FIG. 1. High-power photomicrograph of tubule in the gonad of a patient with Klinefelter's syndrome. Primary and secondary spermatocytes and spermatids are evident in this seminiferous tubule.

chromatin. By tradition, the presence of spermatogenesis would invalidate the female sex chromatin findings. However, Barr studied the sex chromatin in tissue from a case of true hermaphrodism reported by Greene *et al.* (8). The nuclei of the interstitial cells of the testis and of the ovary contained the female-type chromatin, as did the cells of the skin. This is the only other case known to us in which male gametogenesis has been found together with a female-type chromatin.

Histologic study of the gonads removed from each of the 3 Klinefelter patients failed to reveal recognizable ovarian tissue. Furthermore, during an exploratory laparotomy in 1 of these cases, no structures recognizable as internal female genitalia were found. Until further evidence resolves the apparent contradiction of female chromatin in the somatic cells of patients having male-type gametogenesis, it is well to heed Barr's admonition.

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BLOOD CONTENT OF HUMAN PLACENTA*

To the Editor:

The isolation of steroidal and other substances from the human placenta has involved a large amount of work on the part of many investigators. It is well known that the placenta contains large sinuses capable of supporting relatively large volumes of blood. Still, the contribution of the blood is not always properly evaluated.

In a recent study of the steroids present in the placenta (1), a large pool of placentas was collected. The amount of adrenocortical hormones isolated from 9 kilograms of placenta was approximately 85 per cent of that which would be anticipated from an equivalent mass of pregnancy blood (2, 3). In order to determine whether the isolated steroids reflected synthesis or placental concentration of blood steroids, a crude determination of the amount of blood in the average placenta was undertaken.

MATERIALS AND METHODS

Nine placentas were obtained at delivery and frozen in a deep-freeze until the time of extraction. At that time they were finely ground in an electric meat grinder and an aliquot was extracted with distilled water. The extract was analyzed for hemoglobin by the cyanomethemoglobin method of Crosby *et al.* (4), using the Evelyn spectrophotometer. No attempt was made to separate the hemorrhagic blood adhering to the placenta, as this also was to be extracted for steroid analysis. In many of the patients routine clinical hematocrit and hemoglobin determinations had been made, and from these the concentrations of whole blood and plasma in the placentas were estimated (Table 1).

RESULTS

It should be emphasized that these data furnish only a concept of magnitude for the amount of blood and plasma in the placenta. The technical difficulties of a properly controlled analysis would entail such factors as careful removal of "non-placental" blood, washing of the sinuses, separation of fetal and maternal blood, and proper estimation of maternal hematocrit and hemoglobin levels. Even then, it does not necessarily follow that the concentration of erythrocytes in the maternal peripheral blood approximates that found in the placenta. However, the purpose of this investigation was to determine the range of error contributed by blood in the analysis of placental extracts.

From these data it is assumed that the placentas contained approximately 10 per cent plasma (Table 1). The concentration of 17,21-dihydroxy, 20-ketosteroids in pregnancy plasma, as reported independently by two investigators (2, 3) is about 35 μg. per 100 ml. Berliner, Jones and

* This investigation was supported by a research grant, G-3843(C) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

TABLE 1. BLOOD AND PLASMA CONTENT OF HUMAN PLACENTAS

Placenta No.	Wt. of placenta (Gm.)	Hemoglobin content of placenta (Gm.)	Hemoglobin conc. of blood (Gm./100 ml.)	Per cent blood in placenta	Volume plasma* (ml.)
1	230	4.6	15.0	13.5	19.7
2	493	15.8	14.0	23.0	67.7
3	660	9.8	14.0	10.7	42.0
4	391	9.0	14.5	15.9	38.6
5	917	30.3	10.0	33.4	129.9
6	345	7.6	14.0†	16.1	32.6
7	370	8.5	14.0†	16.6	36.4
8	348	5.2	14.0†	10.6	22.3
9	446	8.9	14.0†	14.2	38.1
Total	*4200*	*99.7*			*427.3*

* Plasma content estimated on basis that 14 Gm. of hemoglobin is equivalent to a hematocrit reading of 40.

† Hemoglobin values not available; estimated at 14 Gm./100 ml. blood.

Salhanick (1) found that the substances isolated from placenta that would react positively in the Porter-Silber test used for the estimation of blood steroids were present in a concentration of approximately 100 μg. per Kg. Thus, about 35 per cent of the steroids isolated probably came from the blood accompanying the placenta. In a similar study, Mitchell and Davies (5) found that the blood content of the placental tissue was about 9 per cent.

The placental: whole-blood gradient of these steroids might contribute further information pertinent to the source. The gradient for progesterone is about 20:1 (1, 6, 7); for estrogen, at least 100:1 (5, 8, 9); but for the adrenal-like steroids, about 1:3. In this range the contribution of the peripheral blood should be carefully assessed.

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APPLICATION OF A SIMPLE ION EXCHANGE RESIN TECHNIQUE TO DETERMINATION OF SERUM OR PLASMA PROTEIN-BOUND I^{127} OR PROTEIN-BOUND I^{131}

To the Editor:

Several investigators have used anion exchange resin to separate I^{131}-labelled inorganic iodide from protein-bound I^{131} (PBI^{131}) in human plasma. Scott, Reilly and associates (1, 2) proposed that plasma be shaken with resin. Zieve *et al.* (3) and Fields *et al.* (4) achieved a more satisfactory separation by passing plasma through a 21-cm. column of anion exchanger. We have found that finely divided resin may be used in a convenient and highly efficient column technique to simplify not only determination of PBI^{131}, but also determination of protein-bound I^{127} (PBI^{127}) in the presence of high levels of serum inorganic iodide.

Materials and methods. The anion exchangers used are Dowex 1 or Dowex 2 (×10, 200–400 mesh), prepared in the chloride form. Columns, 3.5 cm. in length and 4 mm. in diameter, are formed by drawing an aqueous slurry of resin into 5.2-cm. lengths of polyethylene tubing, previously closed at one end with glass wool. The open end of the tubing is then closed with glass wool, and the prepared columns stored in distilled water until required for use. (The iodide-exchange capacity of the resin is not diminished after three months' storage in distilled water.)

As shown in Figure 1, the column casings are attached directly to syringes, which are used to draw serum or heparinized plasma through the columns for removal of iodide. A pre-measured sample is drawn from a test tube through the column into the syringe; test tube and column are then washed free of serum by adding 1 or 2 ml. of isotonic saline to the test tube and drawing the saline through the column. (When 1-ml. samples are required, a tuberculin syringe may be used both to draw serum through the column and to measure sample volume. A three-way stopcock can be inter-

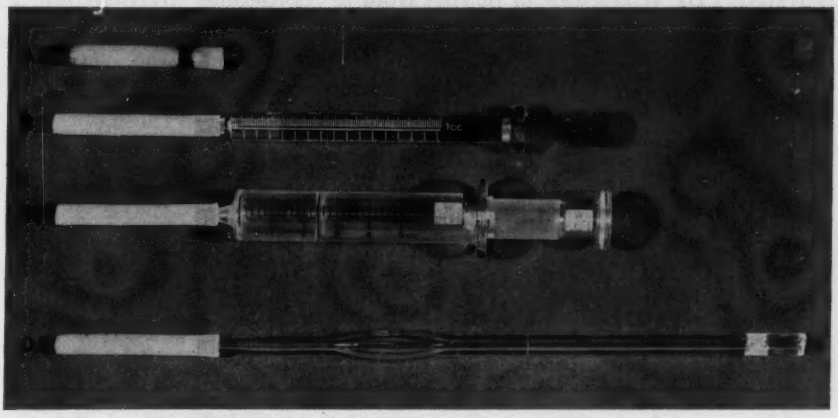

FIG. 1. **A)** Markings on detached resin column indicate position of glass-wool plugs. **B)** Column attached to tuberculin syringe, and **C)** to 5-ml. syringe. **D)** Column attached to serological pipette proved to be less practical.

posed between column and syringe to eliminate the necessity of detaching the column for transfer of the sample or saline.) An alternative method is to draw the sample into the syringe before attaching the column; the sample is then discharged through the column. To obtain samples undiluted by either saline or column water content (approximately 0.2 ml.), the initial 0.5 ml. of effluent may be discarded, and aliquots of required volume pipetted from the remaining effluent. This method yields satisfactory results.

Column characteristics. Samples measuring 2 or 4 ml. were passed through columns at flow rates of 6 to 10 or 4 to 6 ml. per minute, respectively. As shown by I^{131} tracer methods, this procedure removed 99.6 to 99.8 per cent of serum inorganic iodide from samples of pooled or fresh sera containing either physiologic inorganic iodide or 10 *milligrams* per 100 ml. of inorganic iodide added *in vitro*. From samples containing 100 mg. per 100 ml. of added iodide, 99.4 per cent was removed; and from samples containing 7,500 mg. per 100 ml., 90 per cent was removed. The excess exchange capacity or, more specifically, the excess column length indicated by these

values, was found necessary to ensure optimum results at convenient flow rates. Slightly smaller amounts of iodide were removed from serum cooled to 5° C. than from serum at 22° C.

Determination of PBI^{131}. Plasma was obtained from patients twenty-four hours after oral administration of 50-μc. to 250-μc. doses of I^{131} for other diagnostic studies. PBI^{131} concentration was determined by measuring activity in column effluents. PBI^{131} "conversion ratios" were obtained by comparing activity in effluents with activity in the original plasma. Representative PBI^{131} activities in plasma from 1 "borderline" hypothyroid, 2 euthyroid, and 2 hyperthyroid patients were respectively 0.02, 0.07, 0.12, 0.17 and 1.0 per cent of the dose administered per liter of plasma. The "conversion ratios" determined in the same plasma were 3, 11, 41, 73 and 83 per cent, respectively. These results are in accordance with those obtained by other investigators using ion exchange (4) or trichloracetic-acid precipitation methods (5).

Determination of PBI^{127}. By an ashing method similar to that of Barker (6), PBI concentration was determined in serum before and after passing it through columns. Serum protein precipitated in effluents was not washed after decanting the supernatant liquid. It was found that the PBI level in effluents was elevated by not more than 1 μg. per 100 ml. on addition of 1,000 μg. per 100 ml. of inorganic iodide to the original serum. The method was ineffective in correcting spuriously elevated PBI values measured in serum obtained from patients two days after cholecystography. On the basis of these and other experiments which will be reported in a later communication, it is believed that the technique described may provide a simple routine method suitable for determinations of PBI concentration in sera containing large amounts of inorganic iodide resulting from medication or contamination with iodide. (It is probable that protracted extraction of butanol with Blau's reagent can be similarly curtailed in determination of serum butanol-extractable iodine (7).) To date, problems associated with preparing resin completely free of iodide have precluded using ion exchange as a complete alternative to precipitation and washing of serum protein; 0.1 to 0.2 per cent of contaminating iodide in resin is eluted by the passage of 1 ml. of serum and appears in the effluent.

Other applications. Similar columns, prepared from *cation* exchange resin and attached to tuberculin syringes, simplify the method for "bedside" electrolyte determination described by Scribner and Wiegert (8).

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Acknowledgments

The author is indebted to Drs. Morris E. Dailey, Kenneth G. Scott, and Peter H. Forsham for making available the facilities for these studies, to Dr. Chin Tzu Peng for technical advice, to Miss Catherine A. Lambert for her extremely helpful cooperation, and to Miss Violet Hall and Miss Caroline Berg for performing the iodine determinations.

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INTERFERING CHROMOGENS IN THE 17-KETOSTEROID DETERMINATION*

To the Editor:

It was found that the addition of formaldehyde solution to the urine before acid hydrolysis results in some changes in the chromogens usually present in urine samples, so that they no longer are ether-extractable, or are very easily removed from the ether by the NaOH washings. In this way, it was always possible to obtain a perfectly colorless ether extract. In duplicate experiments, it was shown that the values obtained agree satisfactorily with those for non-treated samples. It was concluded, therefore, that the formaldehyde exerted its action exclusively upon the chromogens, and not upon the hormones.

The Holtorff-Koch (1) variation of the Zimmermann reaction was used

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* Presented before the Associação Paulista de Medicina, August 16, 1955. *Rev.paul. med.* November 1955, p. 579.

on an ether extract of HCl-hydrolyzed urine, purified by NaOH and $NaHCO_3$ solutions (Pincus (2)). One milliliter of diluted formaldehyde solution (formalin, 40 per cent, diluted 1 to 5 with water) was enough to prevent color contamination of 50 ml. of urine. The part played by chromogens in the reaction was calculated by running a blank of each extract, in which alcohol was used instead of the *m*-dinitrobenzene sclution. This blank showed 98 to 100 per cent light transmission (against water) for all the formaldehyde-treated extracts. A Coleman-Junior spectrophotometer was used for the measurements.

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CHRONIC ADRENOCORTICAL INSUFFICIENCY AND CARCINOMA OF THE BREAST

TO THE EDITOR:

It has seemed possible to some investigators that steroid hormones of adrenal origin play an etiologic role in the development of carcinoma of the breast. This concept has led to many investigations of the urinary and blood steroids in cancerous patients. Furthermore, total or subtotal adrenalectomy has been advocated for patients with advanced cancer of the breast.

The fact that we have observed the appearance of cancer of the breast in a 32-year-old woman who had been adrenalectomized three and a half years previously for Cushing's syndrome is, therefore, of interest. Because she had chronic adrenal insufficiency and had been maintained on constant small amounts of cortisone and desoxycorticosterone, adrenocortical dysfunction or hypersecretion of normal hormones could hardly have been present when the tumor developed.

L. B. was 28 years of age when first seen by us in January 1952. At this time she presented the classic clinical picture of Cushing's syndrome. The blood pressure was 162 mm. Hg systolic and 116 mm. diastolic. Roentgenograms of the thoracic and lumbar

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spine revealed osteoporosis and compression and anterior wedging of three of the dorsal vertebrae. Results of other laboratory studies were unremarkable except for a diabetic type of glucose tolerance curve. The urinary 17-ketosteroids were 7.8 mg. per twenty-four hours. This finding suggested, but did not prove that the disorder was associated with bilateral adrenocortical hyperfunction rather than with an adrenal tumor.

On February 25, 1952 a right subtotal adrenalectomy was performed by one of us (C.M.S.) without difficulty. Over 90 per cent of the gland was removed and at the time it was questioned whether the small amount of remaining tissue would continue viable. On March 25, 1952 a total left adrenalectomy was performed after preoperative medication with cortisone and desoxycorticosterone. In the postoperative period the blood pressure remained at about 130 mm. Hg systolic and 80 mm. diastolic. Postoperatively the patient was maintained on 25 mg. of cortisone per day, orally.

In an effort to ascertain whether or not she had chronic adrenal insufficiency, cortisone was discontinued on May 10, 1952; four days later she became anorexic, weak and depressed. Her blood pressure fell to 88 mm. systolic and 60 mm. diastolic and she appeared to be seriously ill. Analysis of a 24-hour urine specimen at this time revealed a low level of 17-ketosteroid excretion, *viz.*, 1.2 mg. Cortisone was resumed in a dose of 25 mg. per day and she immediately improved. Thereafter, there was a gradual disappearance of the manifestations of Cushing's syndrome. However increased melanin pigmentation of the skin became evident. In September, menstrual periods began. In October, treatment with 2 mg. of desoxycorticosterone daily (Linguets) was started, in addition to the cortisone. For the next three years her medication consisted of 25 mg. of cortisone and 2 mg. of desoxycorticosterone per day, and she was maintained in excellent health.

In July 1954, the diagnosis of chronic adrenal insufficiency was further substantiated by demonstrating that the low levels of urinary 17-ketosteroids were unaltered by eight-hour infusions of 25 units of adrenocorticotropic hormone daily for three days. When seen again in January and August 1955, she appeared to be in good health.

In early October 1955, *i.e.*, three and a half years after the adrenalectomies, she noted a small mass in the left breast. Surgical removal of the mass was advised and on October 19, 1955 a right radical mastectomy was performed by one of us (C.M.S.). The specimen was examined by Dr. Dann B. Claudon and Dr. Gorton Ritchie, who reported as follows:

"Numerous sections of the biopsy specimen revealed breast tissue altered from normal by the presence of neoplastic epithelial cells derived from ductal epithelium. The medium and small ducts frequently were plugged with solid masses of fairly well differentiated epithelial cells which were quite uniform in size and shape. Microscopic fields of many areas of the biopsy specimen showed that the tumor cells had frequently penetrated the basement membrane of the ducts and had extended into the connective tissues of the breast. Growth in these regions was scirrhous in nature and was not accompanied by an appreciable inflammatory exudate.

Sections of the sixteen axillary lymph nodes revealed the presence of metastatic tumor in each. The tumor had more or less completely destroyed the architecture of the glands and had elicited a considerable degree of desmoplastic reaction. The histological and cytological features of the neoplastic cells in the nodes were similar to those observed in the breast tumor."

Although we can not be certain that this patient did not possess a small fragment of adrenal tissue or some accessory adrenal tissue, we feel that, if present, it was inadequate to maintain life. The darkening of the skin, the low 17-ketosteroid excretion, the prompt appearance of clinical signs

of acute adrenal insufficiency upon withdrawal of replacement therapy, their quick disappearance following small "physiologic" doses of cortisone and desoxycorticosterone, and the lack of a significant rise in urinary steroid excretion upon administration of adrenocorticotropin intravenously, all support the idea that she had either no adrenocortical tissue or an extremely inadequate amount.

The fact that she was kept alive and well for three and a half years with small constant doses of cortisone and desoxycorticosterone suggests that if these hormones played any role with regard to the carcinoma, it was merely one of permitting it to develop. This point of view is supported by Ingle's interpretation (1) of the "permissive" role of the adrenal hormones in stress. Whatever the cause or causes of carcinoma of the breast may be, in our patient they appeared to be sufficient in themselves to initiate the malignant process and to allow it to progress, without any change in adrenocortical secretion. That our case is not unique is supported by the observations (unpublished) of Dr. R. G. Sprague at the Mayo Clinic. He has noted the development of cancer of the breast in 2 women with Addison's disease. Because of these findings, it seems that considerable caution must be exercised when hypotheses of adrenal dysfunction or hyperfunction are considered in relation to the genesis of carcinoma of the breast.

This report bears only indirectly on the problem of the efficacy of bilateral adrenalectomy in the treatment of advanced breast cancer. Although adrenalectomy may exert a transitory beneficial effect in some cases, this response may be due to other consequences of the operation (*e.g.*, the removal of a source of estrogen) rather than to removal of the primary cause of the disorder. Huggins and Dao (2) reported that the better differentiated carcinomas show a high incidence of response to adrenalectomy, whereas the more anaplastic lesions do not. Although Pearson and his associates (3) found a similar correlation in general, there were exceptions to this rule because "some fairly well-differentiated tumors failed to respond and some anaplastic tumors did respond." In our case the tumor was rather well differentiated.

The fact that our patient had Cushing's syndrome prior to the adrenalectomies may also be of interest, but its significance is unknown. In a review of Cushing's syndrome, Plotz and associates (4) reported that 10 per cent of the patients suffered from neoplasms (thymus or pancreas) other than those involving the adrenal or pituitary glands. This may seem to be a rather high incidence of associated carcinoma but it may not be significant. For example, it has been found that in a series of 100 consecutive patients dying of myocardial infarction, 9 per cent had malignant tumors (5); yet so far as is known, there is no relationship between myocardial infarction and malignancy. Thus, there is no secure basis for arguing that

associated neoplastic disease is more or less common in patients with Cushing's syndrome or even in those with Addison's disease. In any event, the present case illustrates that malignant ductal carcinoma of the breast can occur despite the absence or virtual absence of functioning adrenocortical tissue.

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2. HUGGINS, C., and DAO, T. L-Y.: Adrenalectomy and oophorectomy in treatment of advanced carcinoma of the breast, *J.A.M.A.* **151**: 1388 (April 18), 1953.
3. PEARSON, O. H.; WEST, C. D.; HOLLANDER, V. P., and TREVES, N. E.: Evaluation of endocrine therapy for advanced breast cancer, *J.A.M.A.* **154**: 234 (Jan. 16), 1954.
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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should

be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22–27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the field of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Office of the Dean, The University of Texas, Postgraduate School of Medicine, Texas Medical Center, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

FACULTY

A. Albert, M.D., Professor of Physiology, Mayo Foundation; Head, Endocrinology Laboratory, Mayo Clinic.

Willard M. Allen, M.D., Professor of Obstetrics and Gynecology, Washington University School of Medicine.

George W. Clayton, M.D., Instructor of Pediatrics, Baylor Medical School.

Jerome W. Conn, M.D., Professor of Medicine, Director, Division of Endocrinology and Metabolism, University of Michigan.

Frank L. Engel, M.D., Associate Professor of Medicine, Assistant Professor of Physiology, Duke University School of Medicine; Director, Division of Endocrinology, Duke Hospital.

Claude Fortier, M.D., Ph.D., Director, Laboratories of Neuroendocrinology, Blue Bird

Children's Clinic for Neurological Disorders; Associate Professor of Physiology, Baylor University College of Medicine.

James A. Greene, M.D., Professor and Chairman, Department of Medicine, Baylor University College of Medicine, and Clinical Professor of Medicine, University of Texas Postgraduate School of Medicine.

Robert A. Greenblatt, M.D., Professor of Endocrinology, Medical College of Georgia.

John Eager Howard, M.D., Associate Professor of Medicine, Johns Hopkins University School of Medicine.

Charles W. Lloyd, M.D., Associate Professor of Obstetrics and Associate Professor of Medicine, Division of Endocrinology, State University of New York, Upstate Medical Center.

Warren O. Nelson, M.D., Medical Director, Population Council Rockefeller Institute; Professor of Anatomy, University of Iowa (on leave of absence).

Theodore C. Panos, M.D., Professor of Pediatrics, The University of Texas Medical Branch.

Olof H. Pearson, M.D., Associate Professor of Medicine, Cornell University Medical College, Associate Physician, Memorial Hospital.

Rulon W. Rawson, M.D., Executive Officer, Department of Medicine, Memorial Center; Chief, Division of Clinical Investigation, Sloan-Kettering Institute, Professor of Medicine, Cornell University Medical School.

Donald W. Seldin, M.D., Professor and Chairman, Department of Internal Medicine. The University of Texas Southwestern Medical School.

Fred A. Simmons, M.D., Instructor in Gynecology, Harvard Medical School, Associate Visiting Surgeon, Massachusetts General Hospital.

Albert Segaloff, M.D., Director of Endocrine Research, Alton Ochsner Medical Foundation.

Wataru W. Sutow, M.D., Associate Pediatrician, The University of Texas, M. D. Anderson Hospital and Tumor Institute; Associate Professor of Pediatrics, The University of Texas Postgraduate School of Medicine; Clinical Assistant Professor of Pediatrics, Baylor University School of Medicine.

Roy V. Talmage, Ph.D., Professor of Biology, Rice Institute.

J. B. Trunnell, M.D., Head, Section of Experimental Medicine, The University of Texas, M. D. Anderson Hospital and Tumor Institute; Associate Professor of Medicine, The University of Texas Postgraduate School of Medicine.

Henry H. Turner, M.D., Clinical Professor of Medicine, University of Oklahoma School of Medicine; Chief, Endocrine Clinic, University Hospitals.

Judson J. Van Wyk, M.D., Assistant Professor of Pediatrics, University of North Carolina School of Medicine; Assistant Attending Pediatrician, North Carolina Memorial Hospital.

Lawson Wilkins, M.D., Associate Professor of Pediatrics, Johns Hopkins University School of Medicine.

PROGRAM

Monday (A.M.) October 22, 1956

8:00– 9:00 Registration: Place—Clinic Lobby, The University of Texas M. D. Anderson Hospital and Tumor Institute

Introduction to Endocrinology

Chairman—*Dr. J. B. Trunnell*

9:00– 9:40 General Concepts of Endocrinology, including Neurohumoral Interrelationships—*Dr. C. Fortier*

9:40–10:20 Chemistry of the Hormones—*Dr. A. Albert*

10:20–10:35 Intermission

10:35–11:10 Examination of the Patient with Endocrine Disease—*Dr. F. Engel*
11:10–12:00 Application of Laboratory Procedures to the Diagnosis of Endocrine Disease—*Dr. A. Segaloff*
12:00–12:30 Principles of Endocrine Therapy—*Dr. C. Lloyd*
12:30– 2:00 Lunch

Monday (P.M.)

2:00– 5:30 *Panel Discussion with Case Presentations*
Hypothalamic-Adeno-Hypophyseal Function and Disease
1. Physiologic Function of the Adenohypophysis
2. Pituitary Tumors—Anatomical and Physiologic Considerations and Relationship to Hypothalamus
3. Pituitary and Hypothalamic Hyperfunction Syndromes—Acromegaly, Gigantism, Precocious Puberty, etc.
4. Pituitary and Hypothalamic Hypofunction Syndromes—Chromophobe Adenoma, Craniopharyngioma, Sheehan's Syndrome, Diabetes Insipidus, etc.

Drs. A. Albert (Moderator), *F. Engel, C. Fortier, C. Lloyd,* and *O. Pearson*

7:00 P.M. Get-acquainted Cocktail Party—Doctors' Club, Texas Medical Center Library Building. (Sponsored by E. R. Squibb & Sons, Division of Olin Mathieson Chemical Corporation)

Tuesday (A.M.) October 23, 1956

9:00–12:30 *Panel Discussion with Case Presentation*
Spontaneous and Induced Disorders of the Adrenal Cortex
1. Physiological Functions of the Adrenal Cortex
2. Hyperfunction—Cushing's Syndrome
3. Hypofunction Syndromes
   a. Addison's Disease
   b. Iatrogenic Adrenal Insufficiency Induced by Steroid Therapy—Diagnosis and Management
   c. Present Status and Management of Patients Adrenalectomized (or Hypophysectomized) for Cancer, Hypertension, Diabetes or Cushing's Syndrome

Drs. F. Engel (Moderator), *J. Conn, J. Greene, O. Pearson* and *A. Segaloff*

12:30– 2:00 Lunch

Tuesday (P.M.)

2:00– 5:30 *Panel Discussion with Case Presentations*
Disorders of Steroid Hormone Synthesis and Metabolism
1. Normal and Abnormal Biogenesis of Steroid Hormones and the Laboratory Evaluation thereof
2. Virilizing Syndromes due to Disorders of Steroid Synthesis
   a. Adrenogenital Syndrome
   b. Virilization due to Abnormal Ovarian Steroid Synthesis—Arrhenoblastoma, Hilar Cell Tumor, Adrenal Rest Tumor
   c. Differentiation from Other Types of Hirsutism
3. Syndromes due to Abnormalities in Testicular Steroid Secretion
   a. Interstitial Cell Tumor
   b. Adrenal Rest Tumor

Drs. A. Segaloff (Moderator), *R. Greenblatt, W. Nelson, J. Van Wyk* and *L. Wilkins*

Wednesday (A.M.) October 24, 1956

9:00–12:30 *Panel Discussion with Case Presentations*
Disorders of the Thyroid Gland
1. Anatomical and Physiologic Considerations
2. Laboratory Evaluation of Thyroid Function
3. Evaluation of the Patient with Goiter
   a. Colloid Goiter
   b. Adenoma—Single and Multiple, Toxic and Non-toxic
   c. Cancer of the Thyroid
   d. Thyroiditis
4. Hyperthyroidism with Particular Reference to Treatment and Complications
5. Hypothyroidism
   a. Adult Myxedema
   b. Congenital Hypothyroidism

Drs. R. Rawson (Moderator), *A. Albert, J. Trunnell, H. Turner* and *J. Van Wyk*

12:30– 2:00 Lunch

Wednesday (P.M.)

2:00– 5:30 *Panel Discussion with Case Presentations*
Problems of Growth and Development in Children
1. The Endocrine and Non-endocrine Factors Concerned in Growth and Development
2. Abnormalities in Growth and Development
3. Constitutional Variations in the Pattern of Adolescent Development
4. The Types and Diagnosis of Sexual Precocity
5. Ambisexual Precocity (Hermaphroditism)

Drs. L. Wilkins (Moderator), *G. Clayton, T. Panos, W. Sutow* and *J. Van Wyk*

Thursday (A.M.) October 25, 1956

BONES, STONES, AND CALCIUM METABOLISM

Chairman: DR. D. SELDIN

9:00– 9:50 Calcium, Phosphorus and Bone Metabolism and Physiology of Parathyroids—*Dr. R. Talmage*
9:50–10:20 Hypoparathyroidism—*Dr. J. Howard*
10:20–10:35 Intermission
10:35–11:20 Hyperparathyroidism—*Dr. F. Engel*
11:20–12:00 Metabolic Bone Disease—*Dr. J. Howard*
12:00–12:30 Questions and Answers
12:30– 2:00 Lunch

Thursday (P.M.)

THE ENDOCRINE GLANDS AND ELECTROLYTE AND WATER BALANCE

Chairman: DR. J. GREENE

2:00– 2:40 Sodium, Potassium and Water Homeostasis—*Dr. D. Seldin*
2:40– 3:20 The Adrenal Cortex and Electrolyte Balance—Aldosteronism—*Dr. J. Conn*

3:20– 3:50 Vasopressin and Water Balance—Diabetes Insipidus—*Dr. O. Pearson*
3:50– 4:00 Intermission

THE ENDOCRINE GLANDS AND INTERMEDIARY METABOLISM

4:00– 4:30 Endocrine Factors in Diabetes Mellitus—*Dr. J. Conn*
4:30– 5:00 Hypoglycemia—*Dr. J. Howard*
5:00– 5:30 Questions and Answers

Friday (A.M.) October 26, 1956

9:00–12:30 *Panel Discussion with Case Presentations*
Problems of Gonadal Dysfunction (Female)
1. Physiology of Ovarian Function
2. Laboratory Evaluation of Ovarian Function
3. Ovarian Hypofunction Syndromes, including the Menopause
4. Ovarian Dysfunction Syndromes—Menstrual Disorders—Stein-Leventhal Syndrome
5. Use and Abuse of Sex Hormone Therapy in the Female

Drs. W. Allen (Moderator), *R. Greenblatt, C. Lloyd, A. Segaloff* and *F. Simmons*

12:30– 2:00 Lunch

Friday (P.M.)

2:00– 4:00 *Panel Discussion with Case Presentations*
Problems of Gonadal Dysfunction (Male)
1. Development and Physiology of the Testis
2. Laboratory Evaluation of Testicular Function
3. Male Hypogonadism
   a. Primary vs. Secondary
   b. Klinefelter's Syndrome
   c. Cryptorchism

Drs. W. Nelson (Moderator), *A. Albert, J. Howard, F. Simmons* and *H. Turner*

4:00– 4:15 Intermission
4:15– 5:30 *Panel Discussion—Evaluation of Infertility and Failure of Reproductive Efficiency*
1. Male Infertility
2. Female Infertility
3. Habitual Abortion, Toxemia of Pregnancy

Drs. F. Simmons (Moderator), *W. Allen, C. Lloyd* and *W. Nelson*

8:00 P.M. Banquet of the Course, including students and faculty. Doctors' Club, Texas Medical Center Library Building. Cost: $4.25. Tickets will be sold at time of registration
Speaker: *Dr. Warren Nelson*—"Endocrinology and World Population"

Saturday (A.M.) October 27, 1956

9:00–10:30 Demonstration of Laboratory Procedures and Special Techniques and Visit to The University of Texas M. D. Anderson Hospital and Tumor Institute
Drs. R. Greenblatt, J. Trunnell and *G. Clayton*
10:30–10:45 Intermission
10:45–12:30 Questions and Answers—*The Faculty*